Discovery of antifungal and antibacterial volatile natural products and synthetic drug analogs

Von der Fakultät für Lebenswissenschaften

der Technischen Universität Carolo-Wilhelmina zu Braunschweig

zur Erlangung des Grades

eines Doktors der Naturwissenschaften

(Dr. rer. nat.)

genehmigte

D i s s e r t a t i o n

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eingereicht am: 31.01.2018
mündlichen Prüfung (Disputation) am: 27.04.2018
Druckjahr 2018

Vorveröffentlichungen der Dissertation

Teilergebnisse aus dieser Arbeit wurden mit Genehmigung der Fakultät für Lebenswissenschaften, vertreten durch den Mentor der Arbeit, in folgenden Beiträgen vorab veröffentlicht:

Publikationen

T. Wang, P. Rabe, C. A. Citron, J. S. Dickschat, *Halogenated Volatiles from the Fungus Geniculosporium and the Actinomycete Streptomyces chartreusis*, *Beilstein J. Org. Chem.* **2013**, *9*, 2767-2777.

T. Wang, K. I. Mohr, M. Stadler, J. S. Dickschat, *Volatiles from the tropical ascomycete Daldinia clavata (Hypoxylaceae, Xylariales)*, *Beilstein J. Org. Chem*. **2018**, *14*, 135-147.

Tagungsbeiträge

T. Wang, J. S. Dickschat, *New Volatiles from Fungi*. VAAM International Workshop 2013, Frankfurt, Germany (2013).

T. Wang, J. S. Dickschat, *Volatiles from xylariaceous Fungi*. 3rd MINAS Symposium, Warberg, Germany (2014).

T. Wang, J. S. Dickschat, *Investigation of volatile Compounds from Ascomycetes*. 4th MINAS Symposium, Warberg, Germany (2015).

T. Wang, J. S. Dickschat, *An Efficient Approach to the Synthesis of noval Oxazolidinones as potential antibiotic Agents*. MINAS status meeting, Hannover, Germany (2016).

T. Wang, J. S. Dickschat, *Synthesis of potential Bisoxazolidinone Antibacterial Agents*. 5th MINAS Symposium, Warberg, Germany (2016).

Posterbeiträge

T. Wang, J. S. Dickschat, *Investigation of Volatiles from Ascomycetes*. (Poster) VAAM International Workshop 2014, Dresden, Germany (2014).

Acknowledgments

In the first place, I would like to express my appreciation and gratitude to my research supervisor, Prof. Dr. Jeroen S. Dickschat for the chance to start my PhD study in his group and give me interesting research topics. I am also very grateful for his generous, patient and valuable guidance. Without his constant motivation and unconditional support, I could not manage my goal. His true devotion for science has inspired me a lot and helped me overcome all the difficulties and hard moments in the past years. Thank you for taking me to all the conferences and making this thesis possible.

I am deeply indebted to Prof. Dr. Stefan Schulz being referee of this work. I also want to acknowledge Prof. Dr. Matthias Tamm for assuming the examination board.

Hereby I extend my thanks to the current and former members in the Dickschat group for the pleasant working atmosphere in the laboratory and joyful moments in our spare time. Thank all of you: Dr. Ramona Rîclea, Dr. Christian A. Citron, Dr. Nelson L. Brock, Dr. Susanne M. Wickel, Dr. Patrick Rabe, Dr. Khomaizon A. K. Pahirulzaman, Dr. Zhongfeng Ye, Lena Barra, Immo Burkhardt, Ersin Celik, Erik Daniel, Jan Rinkel, Lukas Lauterbach, Etilia Dolja, Tim Klapschinski, Eike Caldeyweyher, Seocho Kim, Noram Kaufmann, Sonia Gibergia, Silvia Klotz, Christopher Jungnickel, Alexander Nikolay, Sarah Schreier, Markus Menke and Benedikt Scholtisek.

I want to thank Prof. Dr. Stefan Schulz and his group for the close cooperation in Braunschweig and for the possibility to the GC/MS machine.

Thanks to my cooperation partners, in particular, Prof. Dr. Joerg Grunenberg from TU Braunschweig for the theoretical calculation of the oxazolidinone derivatives, Prof. Dr. Marc Stadler and Dr. Kathrin I. Mohr from The Helmholtz Centre for Infection Research in Braunschweig for offering the Xylariaceae fungi and examining the bioassays with synthetic substances.

Thanks to all my practical students for excellent synthetic work. These were Madeleine Justianto, Florian Kiene, Andreas Behrens, Carolin Lohmann, Jenny Solarzcek and Jana Maria Adam.

Thanks to the staff of the NMR departments in Braunschweig and Bonn: Dr. Kerstin Ibrom, Dr. Senada Nozinovic, Claus Schmidt, Petra Holba Schulz, Gabriele Krafft, Ulrike Weynand, Karin Prochnicki and Hannelore Spitz. Besides, I want to thank Karin Kadhim, Andreas J. Schneider, Dr. Gregor Schnakenburg, Charlotte Rödde and Dr. Marianne Engeser for the further analytical departments in Braunschweig and Bonn.

Moreover, a special thanks goes to the „Mikrobielle Naturstoffe“ (MINAS) Graduate School for the financial support with the Georg-Christoph-Lichtenberg scholarship from Lower Saxony. I thank Brigitte Brinkmann from FIOC (Freunde des Institutes für

Organische Chemie der TU Braunschweig) and the Gesellschaft Deutscher Chemiker for granting me the financial support for attending conference.

I am highly grateful to all my friends for their kind support and motivation. They are Dr. Ramona Rîclea, Hanzhao Yang, Xu Xing, Srinivasa Rao Ravella, Dr.-Ing. Kin Lien Chee, Eliza Tarcoveanu, Denisa Hathazi, Lena Barra and Paul Barac.

Last but not least, I would like to express my deepest and greatest gratitude to my family, my parents Xinmin Wang and Min Xiao, my sister Yi Wang, my brother-in-law Liang Yao, my little niece Linxuan Yao and my grandparents Zhijian Wang, Hong Yin, and Fuhua Peng, Fangfu Xiao. Thank you very much for the endless love and thoughtfulness all these year.

Table of contents

Abbreviations

Å	Ångström
AA	arachidonic acid
AAC	acetyltransferase
ABC	ATP-binding cassette
ABSSSI	acute bacterial skin and skin structure infections
ACP	acyl-carrier-protein
Ag_2O	silver(I) oxide
ANT	adenyltransferase
AMBER	Assisted Model Building with Energy Refinement
AMEs	aminoglycosides modifying enzymes
APH	phosphotransferase
ATP	adenosine triphosphate
A-site	aminoacyl site
CATs	chloramphenicol acetyltransferases
CCA-Phe	CCA-*N* acetylphenylalanine
CLSA	closed-loop stripping apparatus
DHFR	dihydrofolate reductase
DHPS	dihydropteroic acid synthase
DMAPP	dimethylallyl pyrophosphate
DANN	deoxyribonucleic acid
Dr	diastereomeric ratio
E.coli	*Escherichia coli*
EF-G	elongation factor G
EF-Tu	elongation factor thermo unstable
EI	electron ionization
E-site	exit site
ESI	electrospray ionization
FDA	Food and Drug Administration
fMet	*N*-formylmethionine
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
GTP	guanosine-5'-triphosphate
HCV	hepatitis C virus
HPLC	high-performance liquid chromatography
Hz	Hertz

I	retetion index
iChip	isolation chip
IF	initation factor
IPP	isopentenyl pyrophosphate
IR	infrared spectroscopy
J	coupling constant (in Hz)
LPS	lipopolysaccharide
M^+	molecular ion
MATE	multidrug and toxic compound extrusion
MC	Monte Carlo
MCFA	medium-chain fatty acid
MDR	multidrug resistance
MFS	major facilitator superfamily
MiBIG	Minimum Information about a Biosynthetic Gene cluster
MIC	minimal inhibitory concentration
MRSA	methicillin-resistant *Staphylococcus aureus*
mRNA	messenger ribonucleic acid
MS	mass spectrometry
MVOC's	microbial volatile organic compounds
m/*z*	mass-to-charge ratio
n.i.	no inhibition
$NiCl_2$	nickel(II) chloride
NMR	nuclear magnetic resonance spectroscopy
Nu	nucleophilic
OPLS-AA	Optimized Potentials for Liquid Simulations-All Atom
PAF	platelet-activating factor
PBP	penicillin-binding proteins
pH	potential of hydrogen
Ppm	parts per million
P-site	peptidyl site
PTC	peptidyl transferase complex
R_f	ratio of fronts
RNA	ribonucleic acid
RND	resistance-nodulation-cell-division
r.t.	room temperature
SAM	*S*-adenosylmethionine
SM	starting material
SMR	small multidrug resistance

SPhos	2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl
spp.	*Species pluralis*
TLC	thin layer chromatography
TMP-SMX	trimethoprim/sulfamethoxazole
tRNA	transfer ribonucleic acid
US	the United States
UV	ultraviolet light
Vis	visible light
VOCs	volatile organic compounds
VRE	vancomycin-resistant enterococci
YMG	Yeast and Malt Extract with Glucose

1. Introduction

1.1 History of antibiotic discovery

One of the greatest revolutions in the history of medication is considered to be the introduction of antibiotics, a class of compounds that still play an essential and crucial role in the control of diseases. Mankind benefitted from and misused this form of chemotherapy, leading to the development of antibiotic resistance that has surpassed our knowledge and turned into a tremendously serious issue on a global scale.

Our ancient cultures have been observed to utilize distinct antimicrobial remedies in aspects of curing diseases. In the investigation of fluorescence distribution in human skeletal from ancient Sudanese Nubia (350-550 CE),[1],[2] tetracycline (**1**) in Figure 1 could be traced, as it behaved as an active ion chelator and produced intense yellow-green fluorophors by forming complex calcium and protein compounds.[3],[4] The only explanation for this discovery, is that the deposited food from their daily nutrient was contaminated with the tetracycline-producing *Streptomycetes*.[1] This phenomenon was also found in the samples taken from the femoral midshafts of the late Roman period skeletons from the Dakhlen Oasis, Egypt.[5] An assumption arising from these findings is, that the population infected by transmissible disease in Sudanese Nubian was recorded to be low, whilst no bone infection was revealed in the samples from the Dakhlen Oasis due to the protective effect from oral administration of tetracycline.[5],[6] The incorporation of tetracycline and the hydroxyapatite mineral composition in bones and tooth enamel leaves the obvious evidence for scientific research to tackle the exposure of tetracycline.[7]

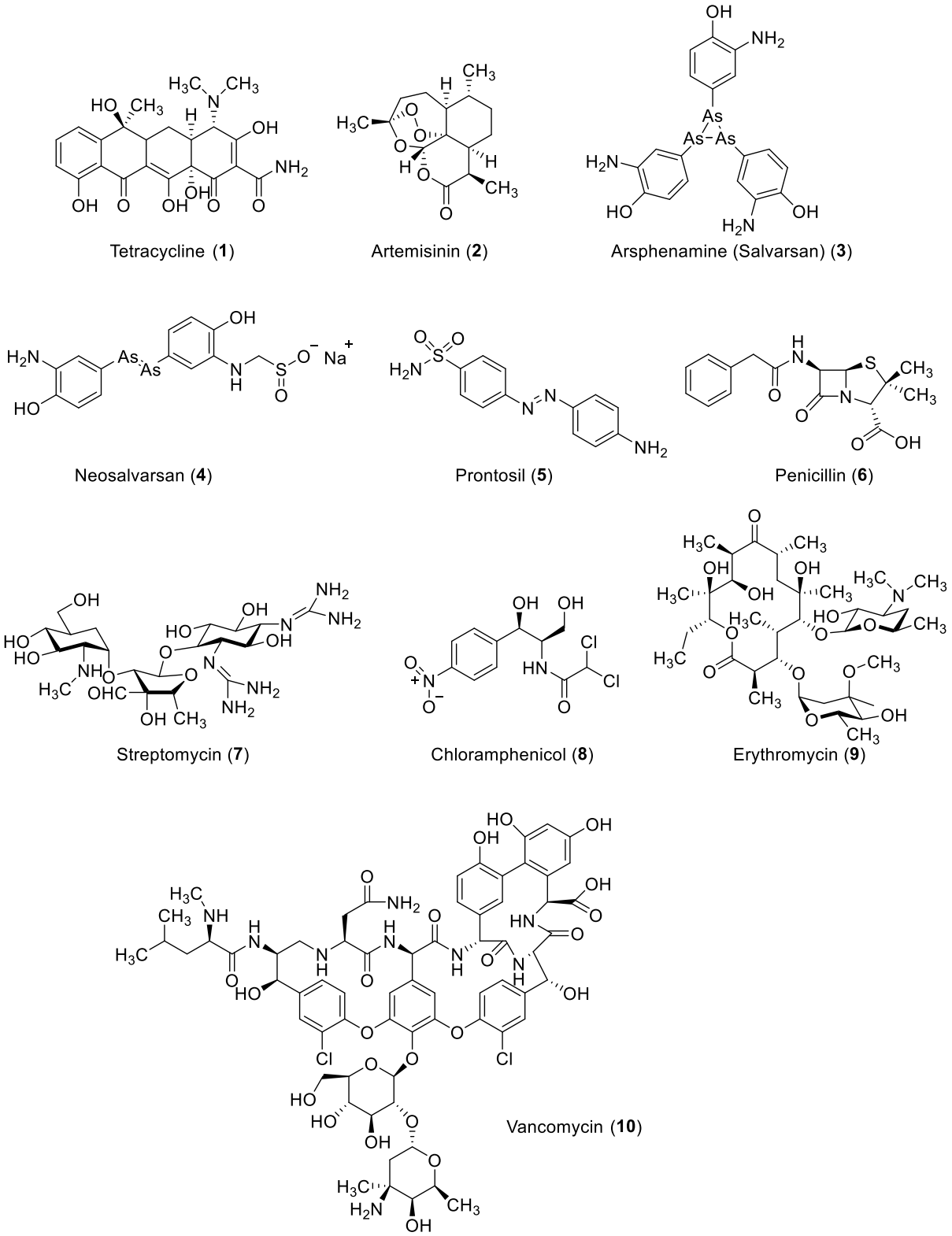


Figure 1 A variety of antibiotics.

However, similar successful examples of tracing other antibiotics in the ancient times are not available when it lacks of evidence. Red soils in the Hashemite Kingdom of Jordan have been used historically for treating

skin infections and diaper rash.[8] They can be purchased in the current pharmaceutical market as a cheap alternative solution.[8] Quite a few antibiotic-producing bacteria and antibiotic remedies were isolated from these soils.[8] For instance, the *Actinomyces* bacteria, *Lysobacter* spp. and *Bacillus* spp., which produce actinomycin C_2 and actinomycin C_3,[8] polypeptide antibiotics that bind to a pre-melted DNA conformation present within the transcriptional complex, preventing the elongation of growing RNA chains.[9] However, these compounds are impossible to be preserved over the centuries.

The traditional or alternative medicine offered a second chance for mining out possible natural antimicrobials with historical relevance, particularly herbal medicine used frequently in traditional Chinese medicine and passed on from generation to generation. Qinghaosu (artemisinin, **2**) is perhaps the most famous anti-malarial drug used by Chinese herbalists for millennia and extracted by Tu Youyou in the 1970s from *Artemisia* plants.[10],[11] An increasing number of antimicrobially active components in the traditional Chinese medicine has been discovered and contributed to the existing arsenal of worldwide medicine. For example, the antimicrobial activity of 20 traditional Chinese medicines against four common oral bacteria, *Streptococcus mitis*, *Streptococcus sanguis*, *Streptococcus mutans* and *Porphyromonas gingivalis*, was validated by Wong's team.[12] Among them, the extracts of *Fructus armeniaca mume* were active against all four bacteria, while other 13 remedies were effective against *Porphyromonas gingivalis*.[12] Another interesting example is the plant of *Pericarpium trichosanthis*, its extracts having a very broad antibacterial spectrum, including 78 nosocomial drug resistant strains like *Acinetobacter baumannii*.[13] This Chinese herbal medicine, the pericarp of *Trichosanthes kirilowii* Maxim or *Trichosanthes rosthornii* Harms (*Fam. Cucurbitaceae*), is traditionally

used for coughs or difficulties in expectorating sputum, dyspnea, chest congestion and other respiratory tract symptoms.[14] Its clinical application is to cure asthmatic tracheitis, cor pulmonale asthma and coronary heart disease.[14] In the analysis of its chemical components by GC-MS, 4-hydroxybenzoic and isovanillic acids were identified and correlated with the distinct antibacterial and antioxidant activity.[13] Further research indicated that the mechanism of action involved the inhibition of the microorganisms' outer membrane functionality and indeed potential in development of new natural antibiotics.[13]

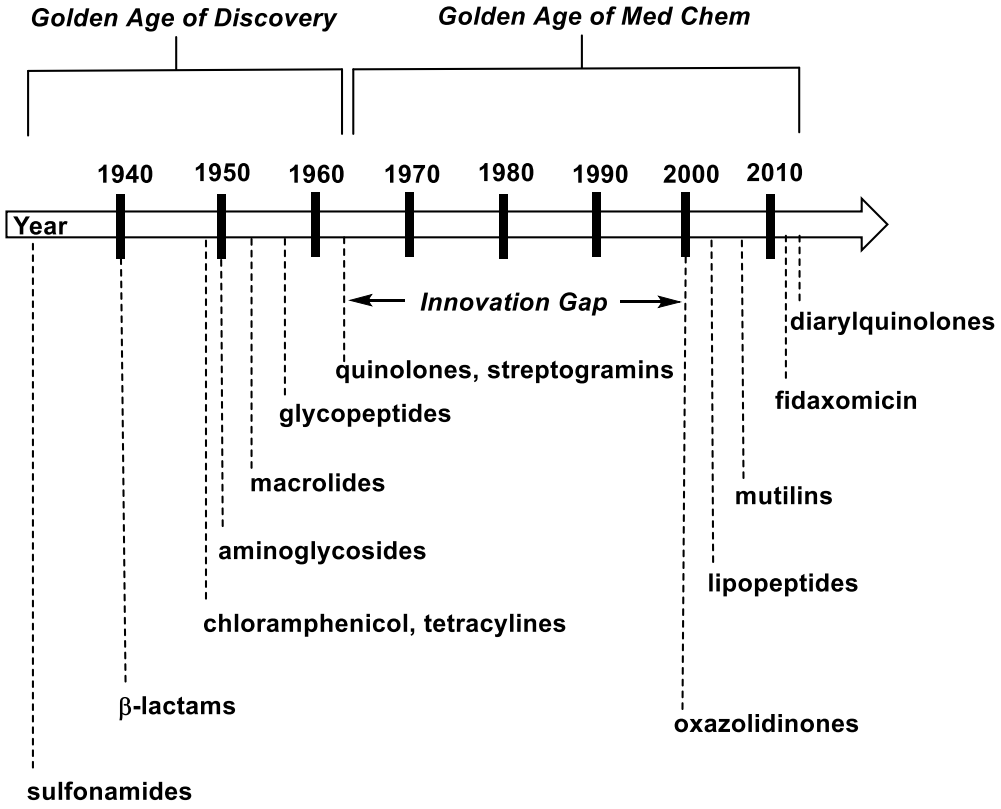


Figure 2 Time scale of the Golden Age of antibiotic discovery (1940-1960) and the Golden Age of antibiotic medicinal chemistry (from 1960 to present). An innovation gap existed between 1962 and 2000, in which no noval classes of antibiotics were explored.[15]

In fact, the modern antibiotic era started with Paul Ehrlich's Salvarsan (**3**). Holding his own invented staining bacteria technique, he performed a large-scale and systematic screening program in 1904 to search a drug

against the sexual transmitted disease syphilis, caused by the spirochete *Treponema pallidum*.[7] Before that, this lethal disease was often handled with inorganic mercury salts, a therapy with a high mortality and poor effectiveness.[7] After testing hundreds of self-synthesized organoarsenic derivatives from a dangerously toxic drug Atoxyl, a compound labeled "606" revealed an extraordinary ability to cure syphilis-infected rabbits and positive clinical results with the restricted trials on patients led to the introduction of Salvaran on the market in 1910.[16] Later, another more soluble and less toxic modified drug Neosalvarsan (**4**) was also marketed and both had success in terms of revenues.[7] However, the mode of action of this drug is still one of the unsolved mysteries though its chemical structure has been already elucidated.[17]

The following discovery of synthetic sulfa drugs, sulfonamidochrysoidine (KI-730, Prontosil, **5**) was also relied on the systematic screening approach invented by Paul Ehrlich.[7] This method has tremendous impact on drug search strategies in the pharmaceutical industry and gave rise to massive production of new drugs, including antibiotics as well.[7] Prontosil was first made by Bayer chemists Josef Klarer and Fritz Mietzsch and later, in 1935, Gerhard Domagk found them active against streptococcal infection in mice *in vivo* but not *in vitro*.[18] Further work indicated that the active part of this drug was in fact its metabolic product, namely sulfanilamide.[19] It might inhibit bacterial growth by interfering with substances, which are essential for the growth of the organism.[20]

The serendipitous discovery of penicillin (**6**) from the mold *Penicillium notatum* by Sir Alexander Fleming in 1929[21] set up an unprecedented gold era for antibiotic research. A Petri dish with growing *Staphylococcus aureus* was accidently contaminated by blue-green mold (a fungus from

the *Penicillium* genus).[21] In addition, the contaminated area was observed to constrain the growth of original microorganism due to the production of specific substances.[21] Fleming obtained these compounds and named them as Penicillin. However, the purification of this antibiotic was done by Howard Florey and Ernest Chain from Oxford after several decades.[22] The clinical usage of penicillin can vigorously lessen the infections caused by Gram-positive pathogens such as *Staphylococcus* and *Streptococcus* species.[23]

In 1944 Albert Schatz isolated another powerful natural product, streptomycin (**7**), obtained from the soil bacterium *Streptomyces griseus* and was the first antibiotic cure for the tuberculosis agent *Mycobacterium tuberculosis*.[24] Together with chloramphenicol (**8**), tetracycline, macrolides (erythromycin, **9**), glycopeptides (e.g. vancomycin **10** and teicoplanin) and other antibiotics, mined out from the rich natural pool, they were so successful to cure and prevent the infections during World War II and considered as miracle drugs for the ultimate medical treatment.[25]

In fact, the first antibiotic drug used in hospital was pyocyanase obtained by Rudolph Emmerich and Oscar Löw from *Bacillus pyocyaneus* (now called *Pseudomonas aeruginosa*) in the 1890s.[26] Owing to its inconsistent performance and toxic preparation procedure, it was eliminated from the market in the end.[26] The following results indicated that the substances with antibiotic property from *Pseudomonas aeruginosa*[27] were two quorum sensing molecules, 2-alkyl-4-quinolone[28] and *N*-(3-oxododecanoyl)homoserine lactone,[29] and one non-enzymatically formed product from the latter molecule, 3-(1-hydroxydecyclidene)-5-(2-hydroxyethyl)pyrrolidine-2,4-dione,[29] respectively.

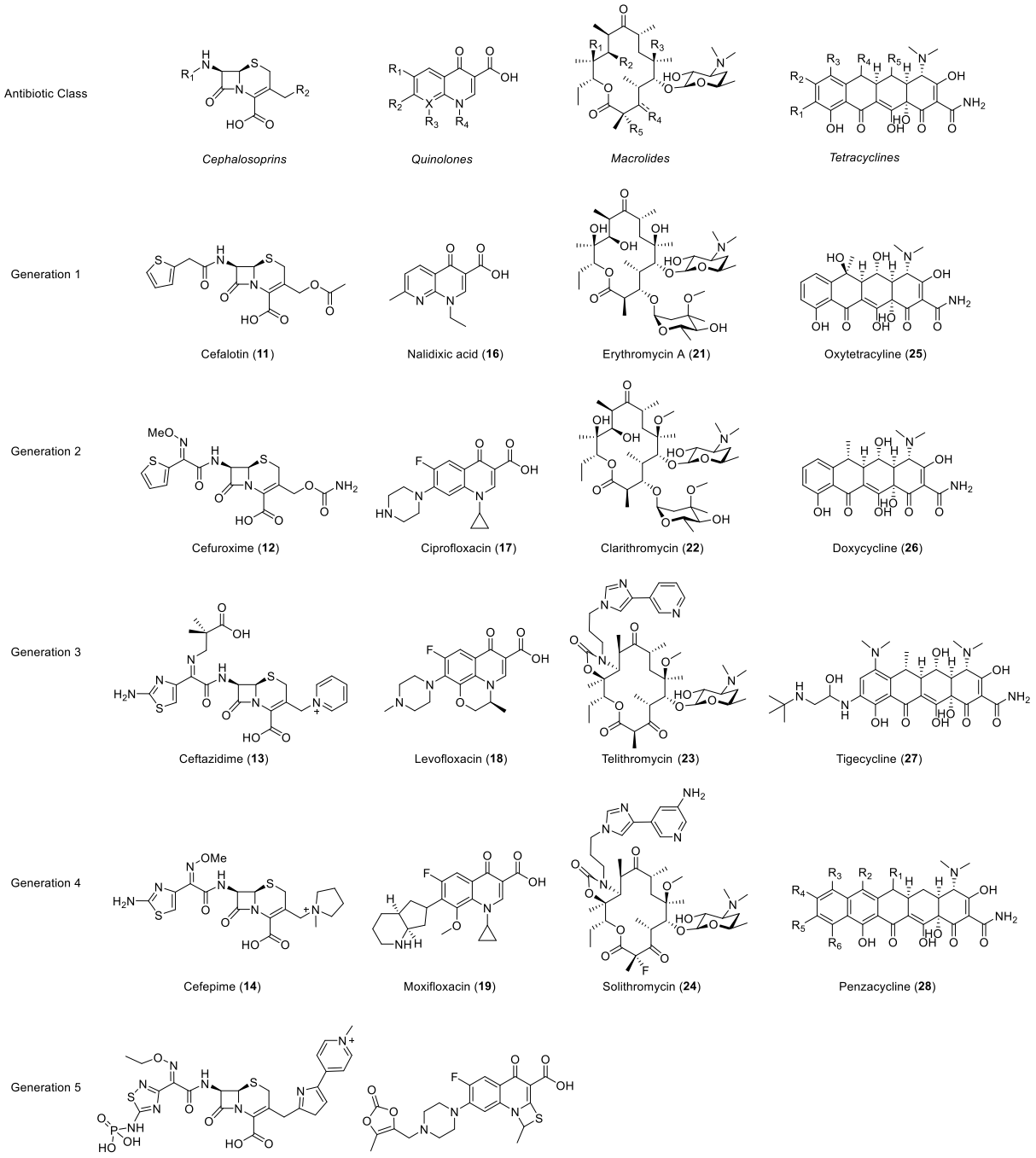


Figure 3 Synthetic tailoring of antibiotic core structures resulted in continuous generations of antibiotic classes in the past 50 years.[15]

Apart from most of natural original antimicrobial agents, man-made ‘magic bullets’ obtained by distinct synthetic approaches were also introduced in clinical therapies. Nalidixic acid (**16**), the first synthesized quinolone antibiotic, was found in 1962 by George Lesher and his

colleagues during the production of chloroquine.[30] It was widely used to treat urinary tract infections in humans.[30]

Till the next new scaffold of antibiotic was developed, in the following four decades the research was only confined to structurally manipulate and optimize the existing antibiotics by synthetic methodologies (Figure 3). Nalidixic acid (**16**) as the first generation of quinolone antibiotics exhibits bactericidal against many different Gram-negative bacteria (e.g. *Escherichia coli*)[31] and some Gram-positive bacteria, including *Bacillus subtilis*.[32] Interestingly, investigation to the mode of action of this agent at different concentrations demonstrated completely opposite results. At reasonable bactericidal concentration, inhibition of deoxyribonucleic acid (DNA) synthesis was observed, instead of ribonucleic acid (RNA) or protein synthesis, whereas at higher concentration, this drugs extends its bactericidal activity and inhibits both RNA and protein synthesis.[31] This agent has been abandoned for its toxicity, low potency and serious adverse effects, specially to infants and young children.[33],[34]

The improvement of this class was continued by the introduction of the second generation. Inspired from the discovery of norfloxacin, with a boarder spectrum of antibacterial activity against both Gram-positive and Gram-negative bacteria exemplified *by Pseudomonas aeruginosa* and *Serratia marcescens*,[35] for which a fluorine atom was installed to the quinolone ring gaining in antibacterial potency,[36] ciprofloxacin (**17**) was developed in 1987 by attaching a different carbon chain on the nitrogen atom compared to norfloxacin.[37] This small change has led it to be the most regularly prescribed second-generation quinolone.[38] Ciprofloxacin has an expanded spectrum of *in vitro* antibacterial activity, particular against Gram-negative bacteria *Pseudomonas aeruginosa* strains,[39],[40] which led to excellent results in many respiratory infection[41] and other

types of infection.[42]-[45] Gram-positive activity however, is poorly represented,[46] for instance, pneumococcal infection[47] which limits ciprofloxacin applications.

Shortly afterwards, third-generation quinolones were synthesized, with the typical Gram-negative activity and atypical intracellular activity but with improved Gram-positive coverage.[46] Levofloxacin (**18**) and ofloxacin were the isomers of quinolone antimicrobial agents obtained by the Japanese pharmaceutical tycoon Daiichi Sankyo.[48],[49] The former compound is a good example for this class, for it is active against a range of Gram-positive and -negative bacteria and atypical organisms[50] and found great acceptance in the treatment of various infections caused by both penicillin-susceptible and -resistant strains of *Streptococcus pneumoniae*.[50] It also shows good tolerability without phototoxicity, hepatic and cardiac adverse events which are quite often seen in some other fluoroquinolones.[51]

The coming fourth-generation agents try to maintain Gram-negative coverage, ameliorate Gram-positive coverage and gain anaerobic coverage.[38],[46] A saturated piperidino pyrrolidine appendage was chemically introduced into the aromatic ring of ciprofloxacin instead of piperazine and one methoxy group was also attached to generate moxifloxacin (**19**) as one of fourth-generation synthetic fluoroquinolone antibiotics.[52] It displays excellent activity against Gram-negative bacilli (Enterobacteriaceae, *Haemonphilus influenzae*, *Moraxella catarrhalis*), enhanced Gram-positive activity against *Staphylococcus pneumoniae* (both penicillin-susceptible and penicillin-resistant strains) and *S. aureus* and ameliorated activity against anaerobes in comparison to ciprofloxacin.[53],[54] It also exhibits better activity against atypical

pathogens and more superior antibacterial ability against *Legionella pneumophila* than erythromycin.[55]

Moreover, the story of development of new antimicrobial agents through the chemical tailoring of main antibiotic core structures has never been completely finished in the past 50 years. Meanwhile, the engine of searching new antibiotic scaffolds is continuously running. An entirely novel synthetically antibiotic, linezolid, was designed in early ‘90s and approved for usage by FDA in the US in 2000.[56],[57] As first-generation oxazolidinone, it possesses potent activity against most Gram-positive bacteria and highly effectively against multi-drug bacteria, including streptococci, vancomycin-resistant enterococci (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA).[58] It is often used to treat complicated skin soft structure infections and hospital-acquired pneumonia through oral and intravenous administration.[59],[60] However, after its commercialization, linezolid-resistant strains of *Staphylococcus aureus*[61] and *Enterococcus faecium*[62]-[64] were discovered clinically. So the possibilities of design and synthesis of novel compounds with improved potency of antimicrobial activities as well as activity against linezolid-resistant strains were explored according to the structural conformation of linezolid. Tedizolid (also named torezolid) is a second-generation oxazolidinone antibiotic and its phosphate derivative was developed in U.S. in 2014 for treatment of acute bacterial skin and skin structure infections (ABSSSI).[65] It exerts 4- to 16-fold greater effectiveness than linezolid against most Gram-positive microorganisms[66],[67] and activity against the atypical *Chlamydia* spp.[68],[69] and certain linezolid-resistant strains of *S. aureus* including chloramphenicol-florfenicol resistant *cfr*-bearing strains.[70],[67] Recently, a novel oxazolidinone resistance gene (optrA) was isolated from *Enterococcus faecium* and *Enterococcus faecalis* in China.[71] This gene

can confer transferable resistance or elevated MICs to oxazolidonones (linezolid and tedizolid) and phenicols (chloramphenicol and florfenicol) when no clinical breakpoints were available.[71]

The research for developing new antibiotics originated from either natural products or synthetic chemicals is still interest triggering. Before investing billions of dollars and time-consuming efforts in drug development, it is practical and highly demanding to fully understand drug-target interactions and the development of the antibiotic resistances in order to manufacture more new approaches to expanding this antimicrobial arsenal.

1.2 Antibiotic modes of action

The majority of antibiotics display their antimicrobial activity at specific sites of microbes or certain metabolic processes, due to their structure feature and affinities to distinct targets within bacterial cells.[72] Two main groups of antimicrobial agents (bactericidal and bacteriostatic antibiotics) can be distinguished from each other according to their different interactions with the bacterial cellular components or system resulting in either induction of cell death or inhibition of cell growth.[73] The mechanism of antibiotic actions can be summarized as follows: inhibition of cell wall synthesis, inhibition of cell membrane function, inhibition of nucleic acid synthesis, inhibition of protein synthesis and blockage of key metabolic pathways.[74],[75]

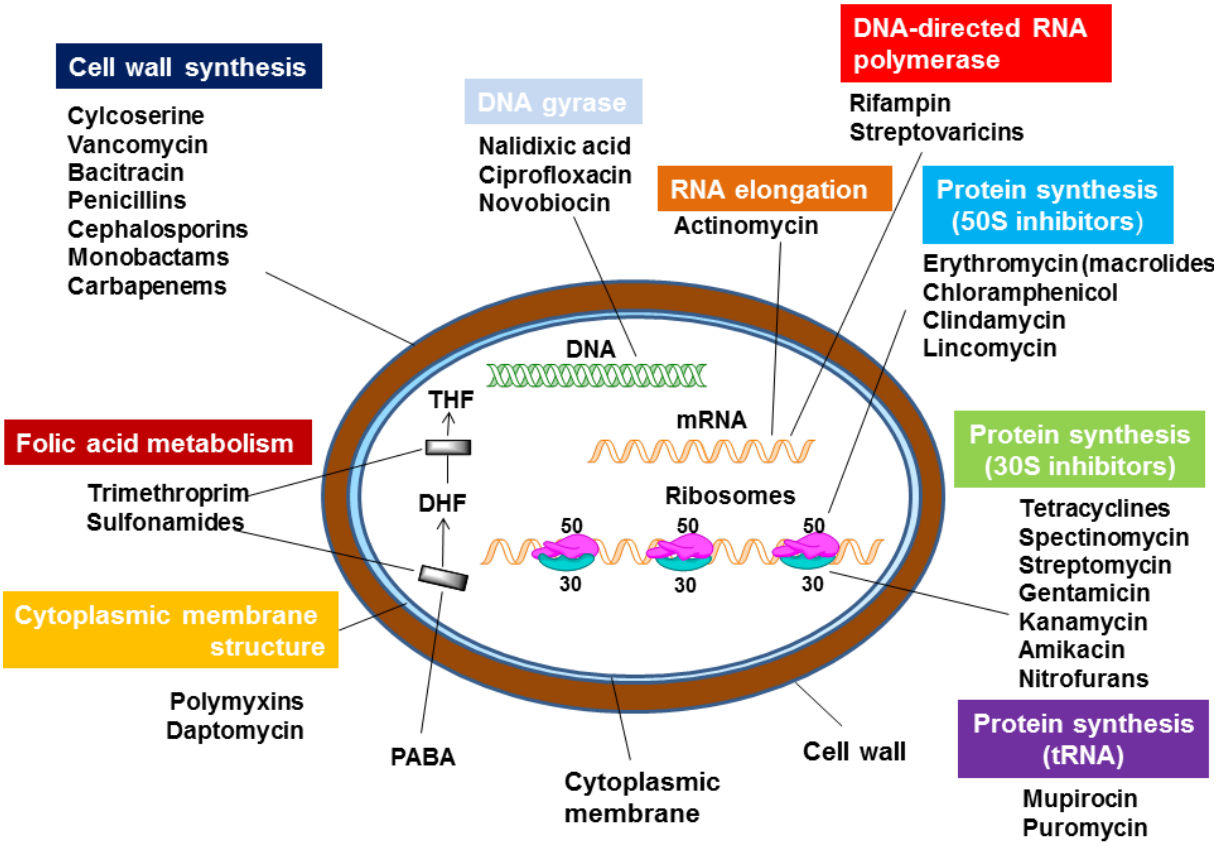
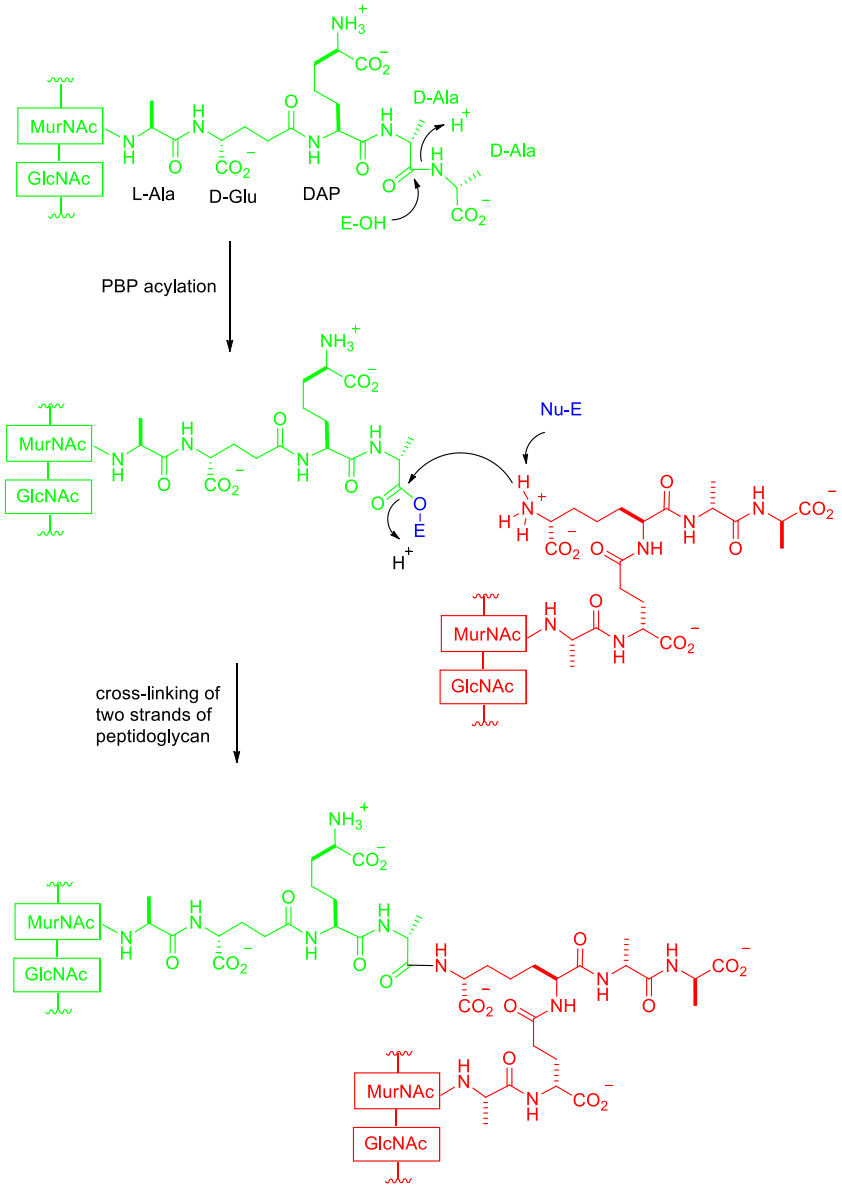


Figure 4 Antibiotic target sites.[75]

1.2.1 Inhibition of cell wall synthesis

Most bacterial cells are encased by firm layers of peptidoglycan (PG, also called murein) which can protect the cells from suffering prevailing osmotic pressures in unfavorable environment.[76] This covalently cross-linked polymer matrix is made up of peptide-linked β-(1-4)-*N*-acetyl hexosamine and its degree is responsible for the structural integrity of the cells.[76],[77] To survive, bacteria have to produce peptidoglycan continuously by transglycosylase and transpeptidase enzymes, which add disaccharide pentapeptides to the glycan strands of existing peptidoglycan molecules and cross-link strands of immature peptidoglycan units.[78] This process is shown in Scheme 1, that the D-Ala terminus of the pentapeptide-functionalized *N*-acetylglucosamine is dismissed in the final cell wall assembly step of Gram-negative and

many Gram-positive bacteria. Subsequently, the acyl part is cross-linked to an amino group in another chain in order to generate the peptidoglycan sacculus as a single polymeric macromolecule.[79]



Scheme 1 Production of Peptidoglycan.[79]

Two classes of antibiotics, β-lactams and glycopeptides, are getting involved in interference with the cell wall biosynthesis in different procedures. A successful cell wall synthesis inhibitor can reshape the cell, induce cellular stress responses and eventually lead to the cell lysis.[80] β-Lactams (penicillins, carbapenems and cephalosporins) can

block the cross-linking of peptidoglycan units through the inhibition of peptide bond formation reaction under the catalysis of transpeptidases (also named as penicillin-binding proteins (PBP)).[78],[81],[82] This takes place in the penicilloylation of a PBP's transpeptidase active site.[73] The group of β-lactam drugs owns the similar structure as the terminal D-alanyl-D-alanine dipeptide of peptidoglycan, therefore it can emulate the action with enzyme in the acylation phase of cross-link formation. In consequence, the enzyme is disabled and lost its ability to hydrolyze the new bond established by β-lactams.[83],[84] On the contrary, actinobacteria-derived glycopeptide antimicrobial agents (exemplified by vancomycin and teicoplanin) act differently, as to interfere with peptidoglycan synthesis in the aspect of binding with peptidoglycan units at the D-alanyl-D-alanine dipeptide as well as blocking transglycosylase and transpeptidase activity.[85] In general, glycopeptides behave as steric inhibitors of peptidoglycan maturation and reduce the mechanical strength of the cells, apart from some that have direct impact on the transglycosylase enzyme.[86] Currently, other antibiotics are introduced in usage, for they are able to restrain the bacterial growth by inhibiting the synthesis (fosfomycin) and transport (bacitracin) of individual peptidoglycan units or affecting cellular structural integrity from penetrating into the cell membrane and prevailing depolarization.[73]

1.2.2 Inhibition of cell membrane function

Bacterial cytoplasmic membranes are generally made up of lipids, proteins and lipoproteins.[87] It serves as a diffusion barrier for water, ions, nutrients and transport systems.[87] In fact, it is considered as a lipid matrix with globular proteins randomly distributed to permeate through the lipid bilayer.[87] Most of antibiotics are working on the mechanism of disorganization of the membranes leading to cell damage.[87] Polymyxin

B and colistimethate (polymyxin E) are two well-known compounds, which bind to lipopolysaccharide (LPS) in the outer membrane of gram-negative bacteria to disrupt both the outer and inner membranes.[88] Principally, it causes disorder of membrane permeability resulting in the leakage of nucleic acids and cations, leading to cell death.[87] Daptomycin, however, depolarizes calcium-dependent membranes that lead to macromolecular synthesis inhibition and disruption of the bacterial membranes.[89]

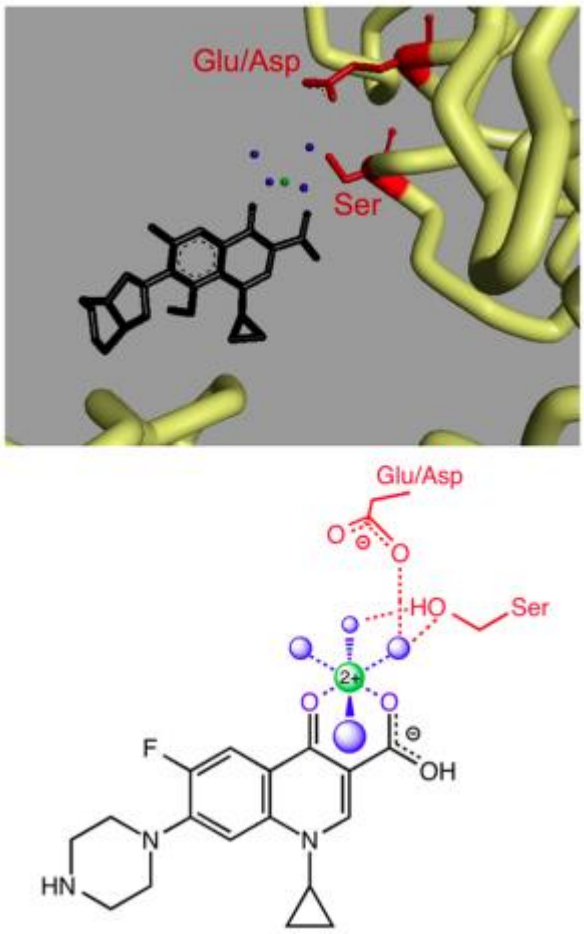
1.2.3 Inhibition of nucleic acid synthesis

1.2.3.1 Inhibition of DNA synthesis

DNA topoisomerase enzymes are responsible for modulation of chromosomal supercoiling via strand breakage and rejoining reactions and further influence DNA synthesis, mRNA transcription and cell division.[73] These enzymes are divided into two types (I & II) only depending on the number of strands of DNA broken in one round of action.[90] They are in charge of the topological state of DNA with cells and indispensable for protein translation and cell replication.[91] In particular, two type II topoisomerases, gyrase and topoisomerase IV, display essential performance in most nucleic acid processes, i.e. controlling levels of DNA under- and overwinding and removing knots and tangles from the bacterial chromosome.[92] Gyrase is the only type II topoisomerase that introduces negative supercoils into DNA in the presence of ATP and also responsible for removing the accumulated torsional stress in front of replication forks and transcription complexes.[93]-[96] Whereas, topoisomerase IV is mainly to remove knots that accumulate in the bacterial chromosome arising from fundamental cellular processes and decatenate daughter chromosomes upcoming replication.[93]-[95],[97],[98] It is noted that two type II enzymes,

topoisomerase IIα and topoisomerase IIβ,[94]-[96],[99],[100] are also encoded in human cells. Despite the similarity in amino acid sequence between them and the bacterial enzymes, the human enzymes unified two individual subunits into a single polypeptide chain after evolution and act as homodimers.[94],[95],[100] The specific amino acid differences between human and bacterial type II topoisomerases make them ideal and attractive targets for antimicrobial agents.[101]

Quinolones are the most successful antimicrobial agents that interfere with the bacterial chromosome by targeting DNA gyrase and topoisomerase IV and disrupting them at the DNA cleavage stage in order to prevent strand rejoining.[102]-[104] The initial study of quinolone targeting revealed that gyrase is the primary drug target and topoisomerase IV is the secondary goal depending on analyzing in which enzyme *Escherichia coli* strains carry the drug resistance mutations.[105] As a consequence, *E. coli* gyrase is more predominantly affected by quinolones than topoisomerase IV[104] and higher levels of gyrase-DNA cleavage complexes in cells are prompted.[106] Strangely, genetic studies in *streptococcus pneumoniae* gave the opposite result that instead of gyrase, topoisomerase IV was actually the primary target for ciprofloxacin.[107] It could be roughly concluded that gyrase is the primary target in Gram-negative organisms, whereas topoisomerase IV is preferentially inhibited by most quinolones in Gram-positive bacteria.[108] Nevertheless, this hypothesis is also not always true. In some cases, gyrase can be the primary target for quinolones in Gram-positive bacteria and distinct quinolones can have different primary targets in given bacteria.[109]-[111] The issue of the targets of quinolone antibiotics is still in dispute and comprehensive evaluation must be done in terms of species by species and drug by drug.[108]



Scheme 2 Quinolone binds to topoisomerase through a water-metal ion bridge.[112] The top sheet demonstrates the crystal structure of a moxifloxacin-stabilized *Acinetobacter baumannii* topoisomerase IV-DNA cleavage complex. The black molecule is moxifloxacin and the noncatalytic Mg^{2+} ion is drawn in green. The four water molecules are colored blue. The yellow backbone is the protein amino acid chain. The side chains of the serine and acidic residues which generate hydrogen bonds with the water molecules in the water-metal ion bridge are painted red.[113] The down panel shows the water-metal ion bridge, displaying the interaction only with protein and not with DNA. Ciprofloxacin is painted in black and the noncatalytic Mg^{2+}, water molecules, and serine and acidic residues are colored as above.[114]

The reaction mechanism depicts as follows: Initially, a pair of staggered single strands break (nicks) under the action of gyrase, topoisomerase IV is covalently bound to the 5’ ends of the cleaved DNA.[115],[116]

Simultaneously, quinolones bind to enzyme-DNA complexes,[117] which can also happen before cleavage of DNA. This binding with mutant gyrase or topoisomerase results in the failure to cleave DNA.[118],[119] Afterwards, another DNA cleavage-associated procedure takes place instead,[117] that replication of the DNA ends in topoisomerases is inhibited by drug,[112] and a variety of drug-mediated new cleaved complexes are created.[120]-[123] Further studies indicated that Ser83 according to *E. coli* GyrA numbering and an acidic residue, four amino acids downstream play an important role in mediation of drug-protein interactions thereby influence stabilization of cleaved complex.[111],[124]-[129] Crystallographic research suggested that a noncatalytic Mg^{2+} ion was chelated by the C3/C4 keto acid of quinolones and coordinated to four water molecules, two of which were bonded to the serine and acidic residues through hydrogen bonds.[112] This existence of water-metal ion bridge was later proven and confirmed that the serine and acidic residues are the anchor points that supply the connection to the enzymes.[113],[130] Thus, the water-metal ion bridge acts as the primary interaction between quinolones and bacterial type II enzymes and provides the most important evidence that quinolones and their enzyme targets are mediated through C3/C4 keto acid of the drug scaffold.[108] In terms of human type II topoisomerases, they lack serine and acidic residues and are unable to interact with quinolones.[131] Therefore, this unique distinction gives the platform for developing new generation of quinolone drugs by discriminating between the bacterial and human enzymes.[108]

After the quinolone-topoisomerase-DNA complex is formed, DNA replication is restrained at the blocked replication forks and result in inhibition of DNA synthesis, thereby leading to bacteriostasis and ultimately death of cells.[103] This reversible outcome can be influenced

by the bacteriostatic concentrations of quinolones.[103],[132] To be noticed, formation of this complexes with DNA by binding topoisomerases with quinolone drugs can cause serious DNA damage to the cells, due to the high distribution of gyrase in the chromosome.[120],[133] Another consequence of the inhibition of DNA synthesis is to cause the DNA stress response (SOS response), where RecA is triggered by DNA damage and auto-cleavage of the LexA repressor protein is promoted to express the SOS-response genes so as to have DNA repair enzymes.[134] To strengthen the potency of the quinolones, preventing induction of the SOS response can be one of the options.[135]

1.2.3.2 Inhibition of RNA synthesis

Rifamycins are a family of semi-synthetic bactericidal antibiotics that interfere with RNA synthesis, similar to the inhibition of DNA synthesis by quinolones, which cause damage on prokaryotic nucleic acid metabolism and bacterial cell death.[136] This class of drugs blocks DNA-dependent transcription by strongly binding to the subunit of a DNA-bound and actively-transcribing RNA polymerase enzyme.[137]-[139] The subunit can be found within the channel generated by the polymerase-DNA complex, where the fresh formed RNA strand arises from.[140] RNA synthesis could not be performed beyond the addition of two ribonucleotides, because rifamycins inhibit nascent RNA strand initialization sterically.[141] However, it functions not by blocking the elongation step of RNA synthesis, but perhaps by interfering with elongation by allosteric modification of the enzyme.[142]

1.2.4 Inhibition of protein synthesis

Protein biosynthesis takes place on large macromolecular ribonucleoprotein complexes (ribosomes) that uses cytoplasmic

accessory factors in an iterative process termed translation.[143],[144] The ribosomes catalyze peptide bond formation and synthesize the polypeptide according to the genetic code of the mRNA.[143] In principle, this process can be summarized in three sequential steps: initiation, peptide elongation and termination.[143] As main field, the ribosome provides optimal condition and conformational structure for performing the translation. It has two unequal subunits (30S and 50S) which establish translation initiation phase and three tRNA binding sites that span both subunits: the aminoacyl tRNA binding/decoding A site, the peptidyl-tRNA binding P site and the uncharged tRNA exiting "E" site.[145] As the active site of the ribosome, the peptidyl transferase complex (PTC) stays at the interface of the A and P sites on the 50S subunit, afterwards the newly synthesized peptides emerge the ribosome through a 100 Å hydrophobic tunnel at the backside of the PTC.[146]

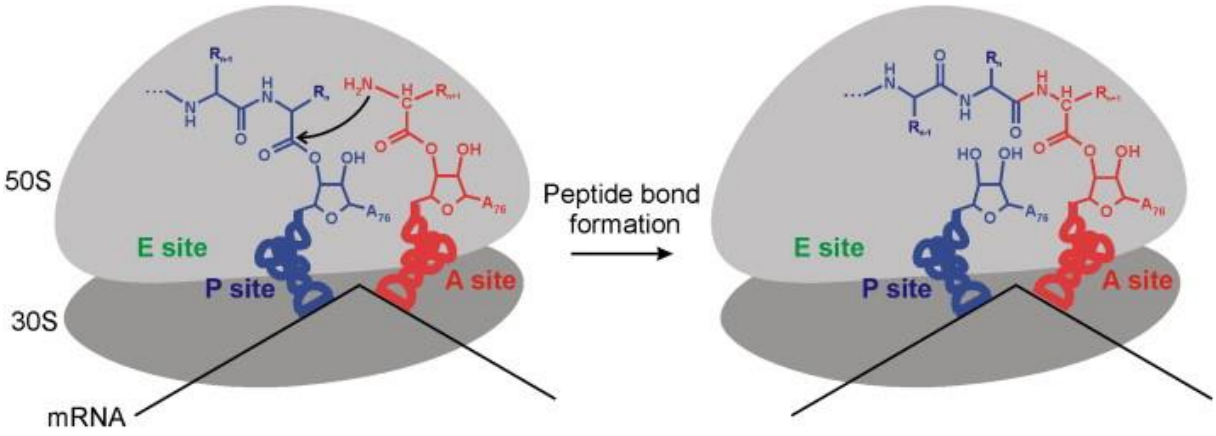


Figure 5 Formation of peptide bond on ribosome. As illustrated in the graphic, the carbonyl carbon of the pept-tRNA in the P site (blue) is attacked by the a-amino group of aminoacyl-tRNA in the A site (red) resulting in a new peptid bond in the A site and a deacylated tRNA in the P site. The 50S subunit is painted in light gray, while the 30S subunit is colored in dark gray.[147]

Initiation of translation is carried out at the first place, where a ternary complex composed of initiation factors (IF-1, IF-2 and IF-3), mRNA, the 30S RNA and initiating *N*-formyl-methionyl-tRNA is formed, and

subsequently binds to the 50S ribosomal subunit to build up a more intact complex.[145] The following step is elongation. The elongation factor Ef-Tu binds with GTP as so to deliver the amino acyl-tRNA (aa-tRNA) into the A site.[148] Incidentally, the ribosome applies decoding process for verifying the accuracy of the incoming aa-tRNA into the A site so that the incorporated amino acids could be proper onto the nascent chain.[148]-[150] Decoding associates with the reversible binding of the anticodon of an EF-Tu-aa-tRNA complex to the codon at the A site of the ribosome.[145] After the accommodation step, conformational modify in EF-Tu and the ribosome results in GTP hydrolysis and the cleavage of EF-Tu.[151],[152] In that case, peptidyl transferase reaction between the peptidyl-tRNA and aa-RNA can occur fluently.[145] Supported by the ratchet-like movement of the ribosome, translocation takes place along the mRNA by one codon leading to the tRNA shifting from the A site to the P site to the E site.[145] Recent studies illustrated the process with idea that 3' end of the aa-tRNA in the A site rotates 180° round a local 2-fold rotation axis in the PTC and migrates the rest of the tRNA molecule into the P sites while peptidyl transfer displays in the PTC at the same time.[145],[153] Finally, a stop codon in the mRNA at the A site terminates the ribosome activity. A variety of cytoplasmic factors including release factor, emancipate the polypeptide and alter the ribosome for recycling.[145]

The drugs that inhibit protein synthesis can be mainly classified into two species: the 50S inhibitors and 30S inhibitors. A variety of antimicrobial agents serve as 50S ribosome inhibitors including the macrolides (e.g., erythromycin), lincosamide (e.g., clindamycin), streptogramin (e.g., dalfopristin/quinupristin), amphenicol (e.g., chloramphenicol) and oxazolidinone (e.g., linezolid).[145],[154] Streptogramins bind to the P site of the 50S ribosome as well as other structurally diverse antibiotics, for instance, macrolides, lincosamides and thiopeptides.[145] Streptogramins

A and B are separately bacteriostatic yet show the bactericidal property if both are used in combination.[155] On one hand, streptogramin A binds to the peptidyl transferase complex in the absence of aminoacyl-tRNAs so that the substance attachment to the donor and acceptor sites are blocked, thereby the early phase in elongation is restrained.[145] On the other hand, mode of action from streptogramin B relies on blockage of protein chains extension to release incomplete peptides, eventually bind to the ribosome at any step of protein synthesis.[145],[155],[156] Oxazolidinones act through a different mode of action. This class also binds to the P site of 50S unit, particularly located near to the 23S rRNA.[145] It interferes with the initiator fMet-rRNA during the formation of the initiating complex, thus influences the formation of the first peptide bond for the mRNA translation.[145],[157],[158]

30S ribosome inhibitors are comprised of tetracycline and aminocyclitol class of antibiotics. Tetracyclines function by inhibition of aminoacyl-tRNAs binding to the ribosome.[159] Spectinomycin and the aminoglycoside family of antibiotics (e.g., streptomycin, kanamycin and gentamicin) belong to the aminocyclitol class and bind the 16S rRNA assembly of the 30S ribosome subunit.[73] Spectinomycin works by disrupting the stability of peptidyl-tRNA binding to the ribosome and blocking elongation factor-catalyzed translocation, yet no influence on protein translation.[160]-[163] However, the interaction between aminoglycosides and the 16S rRNA can cause protein mistranslation instead. The conformational changes in the complex between an mRNA codon and its cognate charged aminoacyl-tRNA at the ribosome were constructed, thereby tRNA mismatching was promoted and leading to protein mistranslation.[164]-[166]

1.2.5 Blockage of key metabolic pathways

Other antibiotics act as essential substrates in the cellular metabolism for the survival of the bacterial pathogens.[72] Bacterial enzymes attach themselves to the antibiotic instead of the regular components.[72] Sulphonamides and trimethoprim stand as typical antimicrobial agents from this group. Both interfere with the folic acid pathway, which plays a significant role in the metabolism of nucleic acid and amino acids.[74] Sulfonamides bind to dihydropteroate synthase, whilst trimethoprim inhibits dihydrofolate reductase. Both enzymes are necessitated for the production of folic acid.[74] With regards to this, the production of nucleic acids (DNA and RNA) and amino acids in bacteria are switched off by sulphonamides and trimethoprim.[74]

1.3 Antibiotic resistance

As an alternative medical treatment instead of normal drugs, the invention of antibiotics transfused new blood into the modern medicine, even once viewed as magic bullets. In 2009, the entire consumption of antibiotics represented 5% of the global drug market and accounted for $42 billion in sales.[167] The upcoming emergence of antibiotic resistance destroyed this elusive utopia and increased the morbidity and mortality rates of infections caused by multidrug-resistant bacteria. Each year, approximately 25,000 patients in Europe suffer from multidrug-resistant bacterial infections and lose their lives,[168] whilst in the United States more than 2 million people obtain infectious diseases arising from antibiotic-resistant bacteria, among which 23,000 could not be cured and died as a result.[169],[170] It has been determined by the World Health Organization as one the three most important public health treats of the 21th century.[169]

As a matter of fact, the coevolution of bacterial resistance and natural antibiotic compounds has been present for billions of years.[171] The recent studies towards the evolution of β-lactamases provided the evidence.[172],[173] However, this has never been an issue blackmailing the human health like nowadays. Because the resistant strains are often less virulent, thus less competitive than the sensitive strains and thereby the selective pressure is minimized.[171] Along with entering the antibiotic era, the evolution was impressively accelerated and production of bacterial resistance was enlarged as well. Penicillin-resistant strains of *Staphylococcus aureus* were detected only 2 years after this antibiotic was introduced clinically.[174] Gradually the situation worsened, as multi-resistance was already observed in enterobacteria during the late 1950s and disseminating since then.[175]

In principle, antibiotic resistance can be divided into three classes, namely, intrinsic, acquired and adaptive.[171] Intrinsic resistance utilizes the inherent structural and functional characteristics from a specific bacterial to resist the activity of antibiotics (Figure 6).[171] For instance, the organisms lack a susceptible target for a specific antibiotic. The biocide triclosan has a broad-spectrum against Gram-positive and many Gram-negative pathogens,[176] but it cannot interfere with membrane growth of the Gram-negative genus *Pseudomonas*. The reason is that an insensitive allele of *fabI* encoded an additional enoyl-ACP reductase, so that the target for triclosan is different as for sensitive species.[177] Another factor for this type of resistance is the composition of the cell envelope, in which the Gram-negative pathogens, like *Pseudomonas aeruginosa* and *Acinetobacter baumannii* hold a semipermeable outer membrane with low permeability.[171] So some agents cannot cross the outer layer and function properly. Vancomycin is such an example as it works well in the Gram-positive bacteria but it is unable to penetrate the

outer layer of Gram-negative species and loses its antibiotic activity.[178] Additionally, the constitutive efflux pumps are present in many bacteria and delivery antimicrobial agents out of the cell.[171]

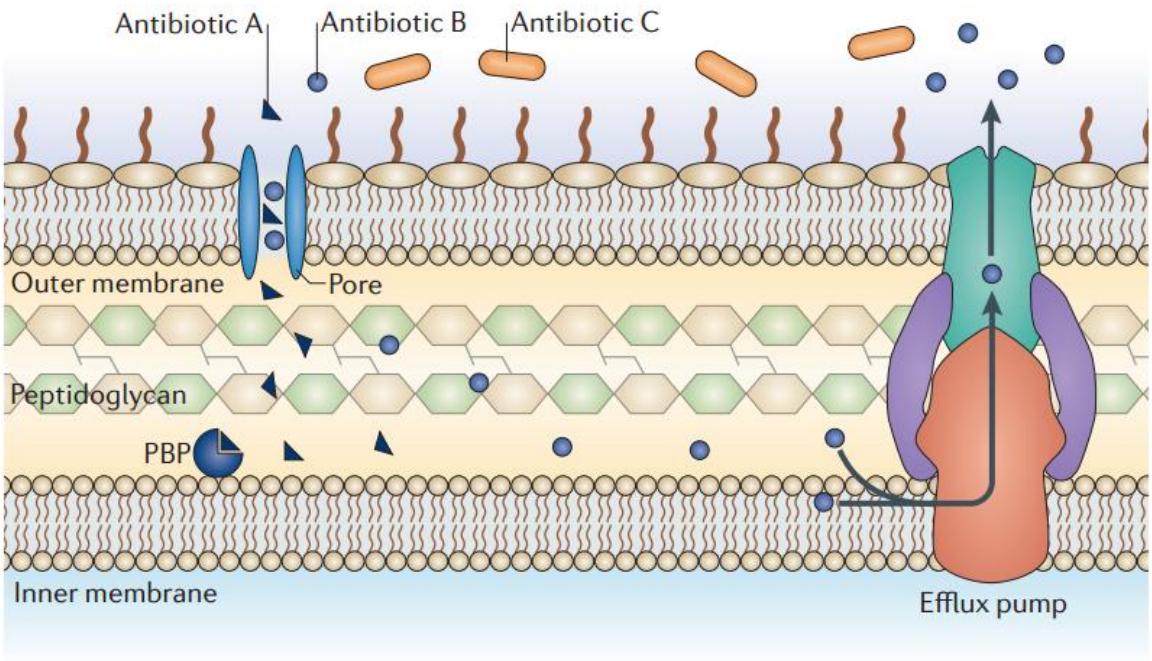


Figure 6 Mechanisms of Intrinsic Resistance. The intrinsic resistance mechanisms are illustrated by the interaction between β-lactam antibiotics and the penicillin-binding protein (PBP). For instance, antibiotic A is succeeded in restraining the peptidoglycan synthesis by penetrating the cell through a membrane-spanning porin protein, thereby binding to PBP. Antibiotic B can perfom the same action, yet it is readily eliminated by efflux pumps. However, antibiotic C cannot transverse the outer membrane to seek the PBP.[176]

Acquired resistance can arise from the susceptible microbes either merged with new genetic material, plasmids (conjugation or transformation), transposons (conjugation), integrons and bacteriophages (transduction) or merely chromosomal mutation leads to cross-resistance.[171],[179] Little changes in mutations can lead to a large improvement in the minimum inhibitory concentration (MIC) and convert low-level resistance into high-level resistance.[171] For example, an additive effect of mutations in the genes *galU*, *neuG*, *mexZ*, and *rplY* on

the resistance of *P. aeruginosa* to the aminoglycoside tobramycin was observed and the individual mutations resulted in merely 2-fold resistance whilst the quadruple mutation created 16-fold more.[171], [180]

In addition, environmental factors, such as stress, nutrient conditions, growth state, or even subinhibitory levels of the antibiotics themselves, lead bacteria to react and alter genes and/or protein expression which produce adaptive resistance.[171] Intrinsic and acquired resistances are stable and can be launched to descendants, whereas adaptive ones are often reversible after removal of the inducing condition.[171]

1.3.1 Genetics of antibiotic resistance

To survive confronting a variety of environmental difficulties, like antimicrobial agents, bacteria can embrace an incredible genetic plasticity.[181] Two major genetic weapons from bacteria can dilute the effect of antibiotic functions, mutational resistance and acquisition of foreign DNA through horizontal gene transfer.[181]

1.3.1.1 Mutational resistance

Mutations in genes are developed among susceptible bacterial cells in order to prevent the cell death from the antimicrobial agents.[181] After a resistant mutant is introduced, the antibiotic takes action and excludes the susceptible population, thereby the resistant bacteria are overloaded.[181] Mechanisms of this sort of resistance is assumed to be the followings: modification of the antibiotic target; the lower uptake of the drugs; activation of efflux pumps against the responsible substances; or global cell adaptions.[181]

1.3.1.2 Horizontal gene transfer

Another common source to manufacture antimicrobial resistance relies on acquisition of external DNA via horizontal gene transfer.[181] The mechanisms of resistance gene transfer between bacteria are comprised of plasmid transfer, transfer by viral delivery, and transfer of free DNA.[179] In general, three main approaches can achieve the gene transformation, including transduction by the way of bacteriophages and integrons, conjugation through plasmids and conjugative transposons, and transformation via incorporation of chromosomal DNA, plasmids into a chromosome.[179] Afterwards, manipulated genes merge with the recipient chromosome by recombination or transposition and result in the changes in the terminal gene sequence.[182]-[184]

1.3.2 Molecular mechanisms of antibiotic resistance

Antibiotic resistance can take place through modifications of the antimicrobial molecule, molecular bypass, active efflux (or decreased permeability) and target modification.[181] The consequences can be diverse and complicated in subjecting different targets. In most cases, bacteria are in possession of distinct resistant mechanisms which interrupt certain class of antimicrobial agents.[185] For instance, the genomic study of a strain of *Acinetobacter baumannii* demonstrated that an 86 kb resistance ‘island’ in chromosome owns 45 resistance genes, among that multiple β-lactamases, aminoglycoside modifying enzymes and efflux systems are contained.[186] Additionally, some mechanisms are spread worldwide, like molecular bypass mechanisms of the global disseminated vancomycin resistance *Enterococcus*.[187]

1.3.2.1 Modifications of the antibiotic molecule

Bacteria apply two strategies for defending themselves against the lethal attack from antibiotics. Bacteria release the enzymes either to inactivate the drug by attaching specific chemical moieties to the complex, or to destroy the compound directly in order to inhibit the antibiotic properties.[181]

Chemical alteration of the antimicrobial molecule performed by appropriate enzymes is a typical acquired antibiotic resistance occurring in Gram-negative and Gram-positive microorganisms.[181] Mainly, it consists of catalyzing acylation (aminoglycosides, chloramphenicol, streptogramins), phosphorylation (aminoglycosides, chloramphenicol), and adenylation (aminoglycosides, lincosamides).[181] The frequent outcome is to enhance the steric hindrance of the molecules so as to decrease the affinity of the drugs to its target, alternately with higher bacterial MIC values.[181]

Aminoglycosides modifying enzymes (AMEs) are one of the typical examples, in which the hydroxyl or amino groups of the aminoglycoside molecule can be covalently changed.[181] According to their different biochemical activities, they can be expressed as aminoglycoside acetyltransferase (AAC), aminoglycoside nucleotidyltransferase (ANT) or aminoglycoside phosphotransferase (APH).[181] The number from 1 to 6 stands for the modification site on the particular carbon of the sugar ring, while the single or double apostrophe depicts the reaction taken place in the first or in the second sugar moiety, separately.[181] Multiple AMEs have been detected and considered as the main mechanism of aminoglycoside resistance nowadays.[181] The distribution of these enzymes are quite different, even from the same family. APH family can be found in Gram-positive and Gram-negative bacteria and changes

kanamycin and streptomycin rather than gentamicin and tobramycin.[181] But then, AAC(6')-I is widely distributed in Gram-negative pathogens including *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter* and influences many aminoglycosides, for instance, amikacin and gentamicin.[188] Adenyltransferases are enabled to impact gentamicin and tobramycin. The genes encoding ANT(4'), ANT(6') and ANT(9') are mostly detectable in Gram-positive bacteria, whereas ANT(2'') and ANT(3'') show up more frequently in Gram-negative microorganisms.[188]

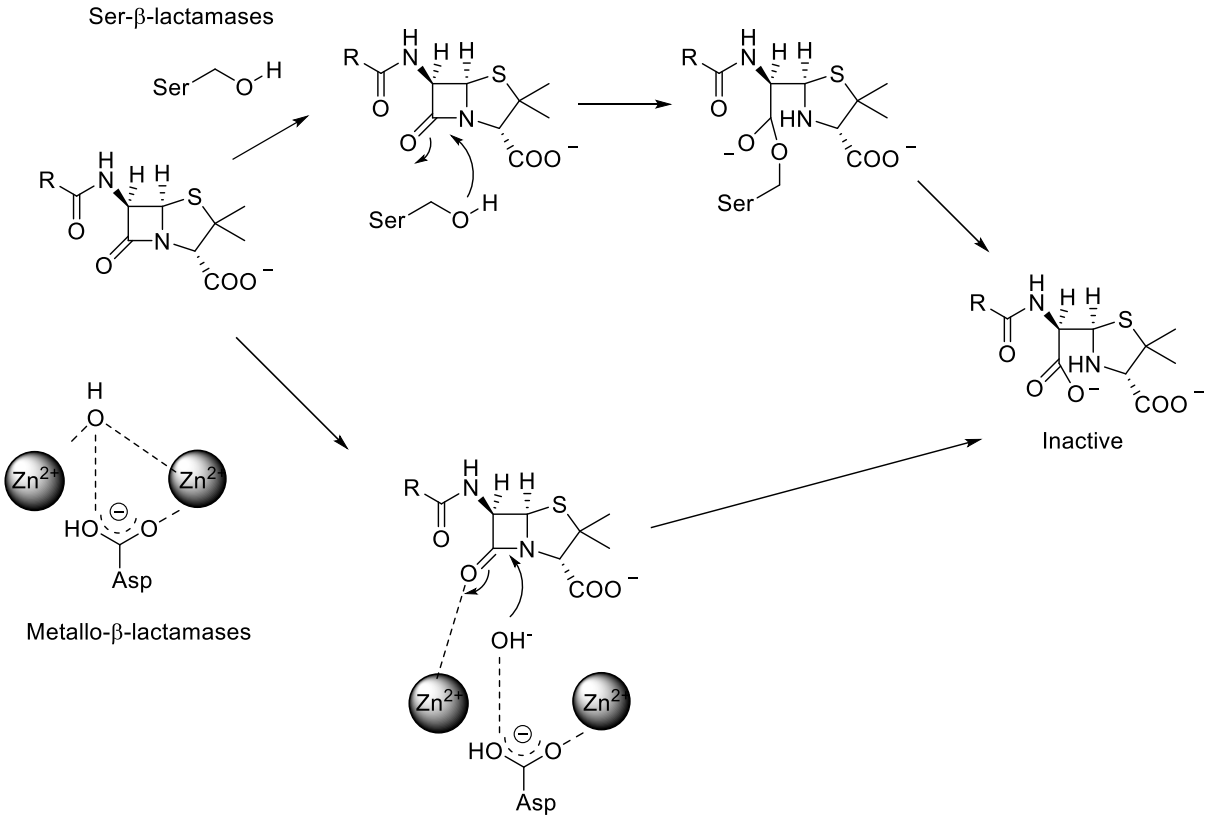
Alteration of chloramphenicol by enzymatic inactivation assumes another representative model, which is conducted by the expression of acetyltransferase termed as CATs (chloramphenicol acetyltransferases).[181] Multiple *cat* genes are mainly located in both Gram-positive and Gram-negative species and cataloged in two major classes. Group A can produce high-level resistance whilst type B only assesses to low-level chloramphenicol resistance.[189]

1.3.2.2 Molecular destruction

β-Lactamases termed as β-lactam resistances are in charge of destroying the antibiotic molecules via breaking the amide bond of the β-lactam ring, hence jeopardizing the effectiveness of the antimicrobial agents.[181] These enzymes were noticed since the 1940s, even one year earlier than the clinical introduction of penicillin and believed to be existed for million years.[190],[191] After penicillin was utilized worldwide, infections caused by penicillin-resistant *S. aureus* became a serious issue in clinic and its resistant mechanism arises from convenient transmission of the plasmid-encoded penicillinase between *S. aureus* strains.[192] Therefore, the manufacture of new β-lactam drugs has considered this as major factor, for instance, ampicillin with wider

spectrum of activity and less susceptibility to penicillinases was developed and produced afterwards.[181] Along with the extensive application of β-lactam antibiotics in medical treatment (penicillins, cephalosporins, carbapenems and monobactams), β-lactamases have become one of the most widespread and clinically notable resistance enzymes.[193]

The mechanisms of β-lactamases can be described in two different ways: a covalent enzyme intermediate is formed or hydrolysis occurs through a nucleophilic water molecule that is metal-promoted (Scheme 3).[194] The former pathway is performed by catalytic Ser, which is functionally analogous to Ser proteinases.[185] Its hydroxyl group attacks the electrophilic carbonyl carbon on the β-lactam ring.[185] As a result, a covalent enzyme intermediate is afforded followed by a rapid hydrolysis leading to the opened lactam ring and inactivation of the antibiotics.[185] The second hypothesis depicts that the metallo-β-lactamases manipulate a water molecule activated by 1-2 active site Zn^{2+} atoms to attack the β-lactam ring directly.[185]

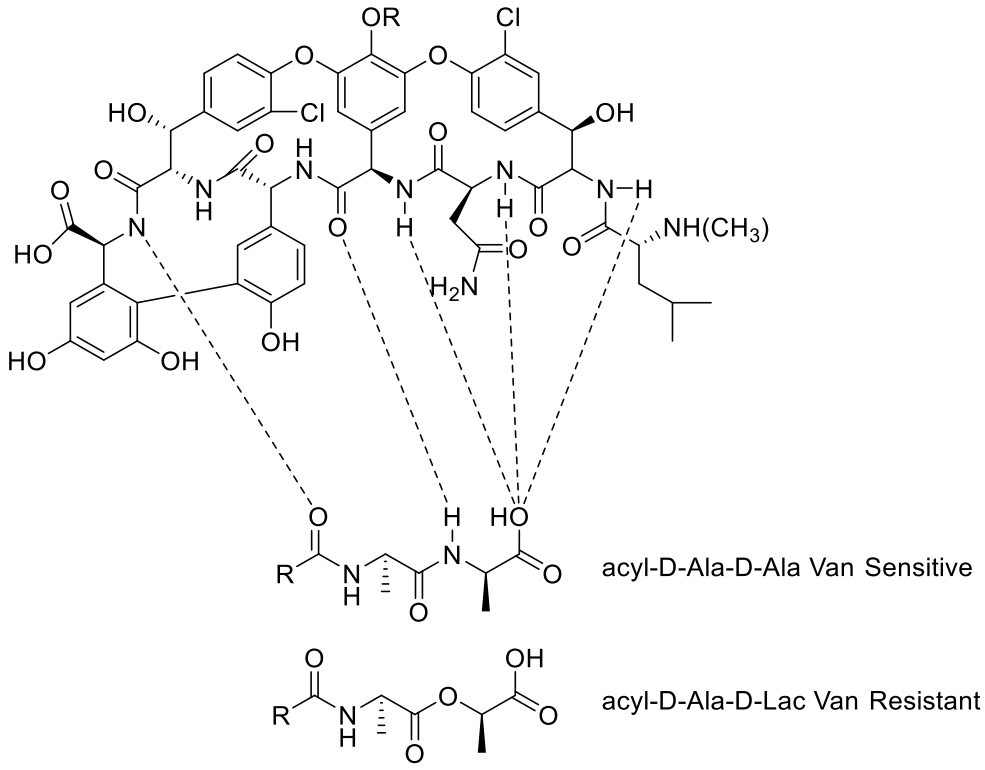


Scheme 3 Two distinct mechanisms of Ser and metallo-β-lactamases.[185]

1.3.2.3 Molecular bypass

Bacteria are able to evolve new working sites which share very similar biochemical functions as the original targets meanwhile are not prohibited by antimicrobial agents.[181] This replacement or bypass mechanism can acquire the effective resistance to the drugs.[181] Vancomycin resistance in enterococci stands for a classic instance from this group. In principle, vancomycin forms a complex by binding the acyl-D-Alanyl-D-Alanine terminus of the developing peptidoglycan which assembly of the bacterial cell wall.[181] A series of five hydrogen bonds is observed in this complex.[195] However, in the resistance mechanism, the isosteric depsipeptide acyl-D-Alanyl-D-Lactate replaces the acyl-D-Alanyl-D-Alanine binding to the antibiotic instead.[181] The new ester linkage abolishes a key H-bond donor and brings in electronic repulsion that weakens the antimicrobial binding.[181] Nine enterococcal *van* gene

clusters (*vanA-vanN*) have been found in soil organisms including *Paenibacillus thiaminolyticus* and *P. apiares*.[196] The *vanADLM* clusters generate precursors ending in D-Lac while D-Ser-ending peptidoglycan is afforded by the *vanCEGN*.[181]



Scheme 4 Vancomycin and other glycopeptide antibiotics bind to the acyl-D-Ala-D-Ala terminus of growing peptidoglycan chains and their precursors with five hydrogen bonds. Resistance occurs with an isosteric replacement of the acyl-D-Ala-D-Ala with acyl-D-Ala-D-Lactate and leads to the loss of one hydrogen bond instead of original solid five hydrogen bonds.[181],[185]

Another well-described strategy is to enlarge the production of antibiotic targets in order to overwhelm the antibiotics.[181] Development of resistance to trimethoprim/sulfamethoxazole (TMP-SMX) is a typical example of this mechanism. Bacteria overproduce the dihydropteroic acid synthase (DHPS) and dihydrofolate reductase (DHFR) via mutations in the promoter region of the DNA.[181] The former synthesizes dihydrofolate from *para*-aminobenzoic acid.[181] This procedure is inhibited by sulfamethoxazole. Another enzyme catalyzes the production

of tetrahydrofolate from dihydrofolate, which is constrained by trimethoprim.[181] These massively manufactured enzymes causes overwhelmed difficulties for the antibiotics, preventing inhibition of folate production.[197],[198]

1.3.2.4 Efflux

1.3.2.4.1 Reduced permeability

Many antibiotics have to penetrate the outer and/or inner membrane to reach intracellular or periplasmic bacterial targets, particularly Gram-negative bacteria, possessing a less permeable outer membrane compared to Gram-positive bacteria.[181],[199],[200] By using water-filled diffusion channels termed as porin proteins, these hydrophilic antibiotics can enter the membranes, such as β-lactams, tetracyclines and fluoroquinolones.[201] Meanwhile, vancomycin has no activity against Gram-negative pathogens due to its inability of penetrating the outer membrane. Decreasing the permeability of the outer membrane can be a strategy for bacteria to cope with the antibiotics.[181],[202] The key step is to alter the porins or replace the porins with more-selective channels.[176] Porins can be divided into different types based on their structures (trimetric or monomeric), selectivity and the expression regulation.[181] Modification of porins takes place in three distinct steps, including changing the type of porins expressed, varying the expression of porins and jeopardizing the porin function.[181] However, all these procedures can lead to low-level resistance and often incorporate with other resistance mechanisms, like activating efflux pumps.[203] To date, reductions in porin expression of Enterobacteriaceae, *Pseudomonas* spp. and *Actinetobacter* spp., play an essential role in resistance to carbapenems and cephalosporins. Both resistances were often considered being mediated by enzymatic degradation.[204]-[208]

1.3.2.4.2 Increased efflux

Bacteria are able to transport the toxic compounds out of the cell via efflux pumps and this action is associated with the intrinsic resistance of Gram-negative bacteria to the drugs which are used to cure the Gram-positive bacterial infections.[176],[181] The earliest observation can be traced back to 1980s when tetracycline was pumped out of the cytoplasm of *E. coli* by an efflux system.[209] Afterwards, intensive research was focused on the characterization of distinct classes of efflux pumps in both Gram-negative and Gram-positive microorganisms.[181] These efflux pumps could be either narrowly substrate-specific (for instance, the tet pumps for tetracycline and mef genes encoding pumps for macrolides in pneumococci) or ship a wide variety of substrates out of the cells, termed as multidrug resistance (MDR) efflux pumps.[210] Substantial antibiotics are suffering from this sort of resistance, in particular, protein synthesis inhibitors, such as fluoroquinolones, β-lactams, carbapenems and polymyxins.[181] There are five main types of efflux pumps that fulfill the assignment, including the major facilitator superfamily (MFS), the small multidrug resistance class (SMR), the resistance-nodulation-cell-division class (RND), the ATP-binding cassette class (ABC), and the multidrug and toxic compound extrusion class (MATE).[181] They differentiate each other according to their structural conformation, energy source, extruded compounds and bacterial organisms that they locate.[211]

Among them, the RND family of MDR efflux pumps is considered the most important and clinically relevant MDR efflux transporters in Gram-negative pathogens.[176] When overexpressed, RND pumps would start to remove massive substrates out of the bacterial cell, including antibiotics (tetracyclines, chloramphenicol some β-lactams, novobiocin, fusidic acid

and fluoroquinolones) and toxic compounds (bile salts, cationic dyes and disinfectants).[181],[211] The most well-studied RND pumps are AcrAB-TolC from *E. coli* and MexAB-OprM from *P. aeruginosa*.[185] Crystal structures suggest that the RND systems embrace a cell membrane spanning pump (AcrB, MexB), an outer membrane pore (TolC, OprM) and a periplasmic adapter protein (AcrA, MexA) that links the former two.[185] Further understanding showed that AcrB has two distinct binding pockets (distal and proximal) which can transport dissimilar compounds with different sizes and properties, which results in broad-spectrum antimicrobial resistance,[181],[212]-[216] subsequently a trimer rotation mechanism supports the exit of molecules through the outer membrane pores.[185]

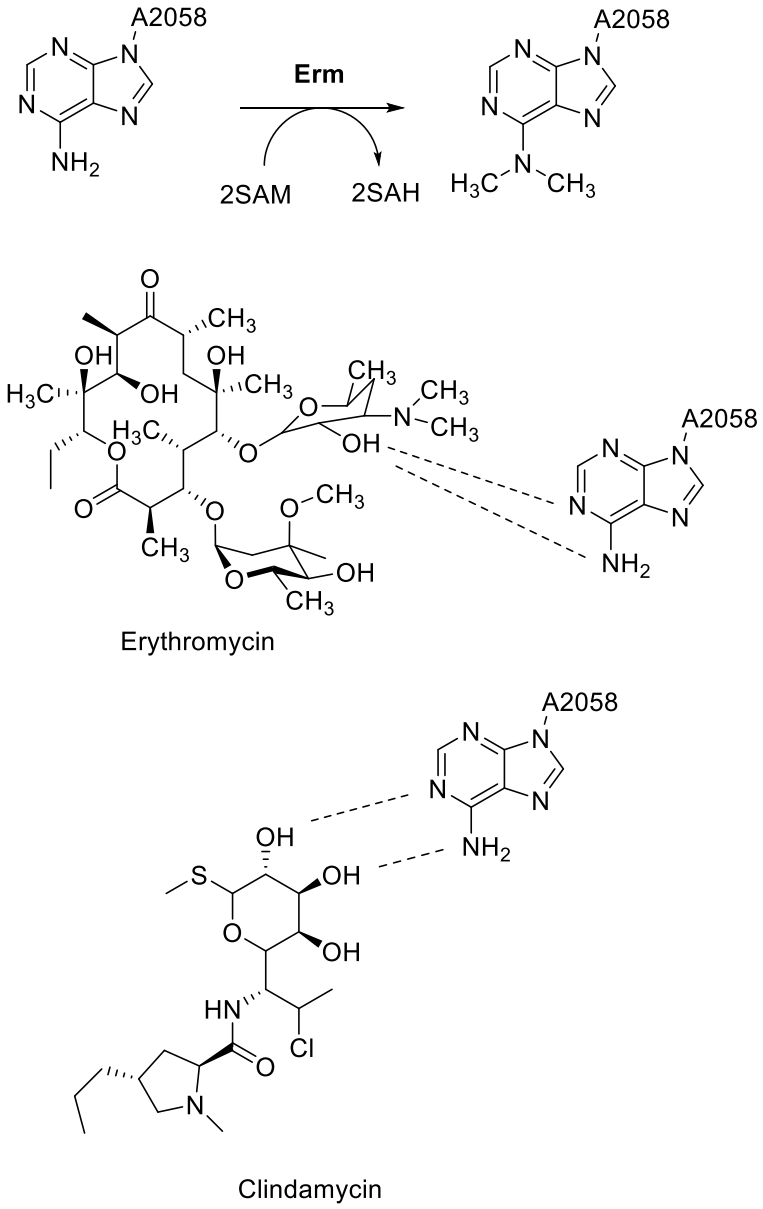
1.3.3 Protection of targets

Several drugs are disrupted by this mechanism including tetracycline (Tet[M] and Tet[O]), fluoroquinolones (Qnr) and fusidic acid (FusB and FusC).[181] For the tetracycline resistances, Tet(M) was found initially in *Streptococcus* spp. and Tet(O) in *Campylobacter jejuni*, later both are observed in different bacteria as well as in several plasmids and broad-range conjugative transposons.[217] They are associated with the translation factor superfamily of GTPases and serve as homologues of elongation factors (EF-G and EF-Tu) in protein synthesis.[181] They incorporate within the ribosome and prevent tetracycline from binding to its target in a GTP-dependent manner.[181] The whole process can be depicted as following: Tet(M) straightly extrudes and liberates tetracycline from the ribosome via a synergy between the domain IV of the 16S rRNA and the tetracycline binding site.[218] As a consequence, the ribosomal conformation is changed and rebinding to the antibiotics is thereby avioded.[218] Tet(O) performs a similar mechanism and its

substitution of tetracycline in the same ribosomal space revises the geometry of the antibiotic binding site so as to replace the antibiotic and restart protein synthesis .[219]

1.3.4 Target modification

Modification of the molecular target is one of the most common mechanisms of antimicrobial resistance. It can be achieved from point mutations in selected genes leading to small resistances, which are harmless to microbes.[185] For instance, single mutations in target genes *gyrA* can result in high level resistance to fluoroquinolone antibiotics such as ciprofloxacin.[185] Mutation of Ser to an amino acid with a heavy side chain (Leu, Trp, Ile) at position 83 or Asp87 to Asn, Tyr, or Gly takes place in fluoroquinolone resistant GyrA giving rise to higher level of resistance.[185],[220]



Scheme 5 Erm methyltransferases alter A2058 chemically so as to interrupt the interation with antibiotics sterically and electronically, thereby resistance occurs. The methyl donor SAM is turned into SAH (*S*-adenosylhomoserine).[185]

On the other hand, enzymatic transformation of the binding sites can also promote antibiotic resistance. Ribosome methyltransferase is one of the best characterized examples of this resistance. The Erm (erythromycin ribosomal methylation) enzymes are able to modify the 23S rRNA of the 50S ribosomal subunit at position A2058 in *E. coli*.[181],[185] Usually, it affects the function of three structurally dissimilar types of antimicrobial agents, the macrolides (e.g. erythromycin),

lincosamides (clindamycin) and type B streptogramins (e.g. quinupristin).[185] Despite the vast diversity in structure, they are all binding to the peptide exit tunnel region of 50S ribosomal subunit.[185] A2058 builds the direct or indirect connections with the drugs via the exocyclic amine of the purine ring, N6. Mono- or dimethylation of N6 is carried out under catalyzed *S*-adenosylmethionine (SAM) by Erm methyltransferases.[185] The outcome is to lose H-bond properties and impair the antibiotic binding sterically.[185] Sharing the similar strategy, methyltransferases can also modify A1408 or G1405 of the 16S rRNA of the small ribosomal subunit and turn out to be the strongest obstacle for aminoglycoside antibiotics over the decades.[221]

1.4 Approaches to development of novel antibiotics

For quite a long period, plants were considered as the most generous and abundant sources of natural medical products.[222] Indeed, a vast amount of active substances has been continuously isolated from them.[222] Most of antimicrobial compounds extracted from plants are phenols or oxygen-substituted derivatives, which are often used by plants to defense the predation from microoganisms, insects, and herbivores.[223],[224] For instance, the terpenoid capsaicin from chili peppers contributes to the plant flavor and similar herbs and spices from mankind's season food are also able to isolate useful medicinal compounds.[224] Later, the first synthetic antimicrobial agents were invented by screening libraries of chemicals in specific dyes.[23] Afterwards, the concept that bacteria and fungi can release the secondary metabolites with high potency and minimal toxicity towards treating bacterial infections in humans was fully accepted and changed the previous perspective.[23] Selman Waksman applied this platform for screening soil-dwelling bacteria and particularly the spore-forming

actinomycetes in order to produce the active compounds that perturb the growth of microbes.[225] This empirical method was referred to Waksman platform.[225] Approximately, tens of millions of soil microbes have been screened by this effective and simply platform in the golden era.[226] As a result, most of the existing natural antibiotic scaffolds were discovered during that period.[23] It also contributed to understanding the minimal inhibitory concentration (MIC) of a compound that is capable of preventing the cell growth *in vitro*.[23] The restriction of this platform was showed up slowly by the mid-1960s, for microbial evolution in designed circumstance propelled the production of unplanned metabolites with unfavorable medicinal properties.[23] The resistance issue stemmed from the horizontal transfer of resistance genes between bacteria or chromosomal mutation became critical as well.[23] The ensuing generation of new antimicrobial agents was mostly innovated from the existing natural scaffolds via chemical modification.[23] The whole strategy can be summarized as bioactivity-guided screening, classical pharmacology, forward pharmacology[227] or phenotypic drug discovery.[228] Potential substances were screened for their bioactivity in whole cell-assays in the absence of the drug target.[225] The research to the target and the mode of action of the compound was accomplished after its biological activity was proved.[222]

The second approach is termed as chemical screening in the purpose of identification of novel structurally dissimilar compounds in the absence of considering the bioactivity.[222] These compounds can be launched from either biological source (e.g. bacterial secondary metabolites) or from chemical libraries.[222] Sophisticated analytical methodologies are involved into the structure elucidation, including high-performance liquid chromatography (HPLC), mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR).[222] With the effort of large data-bases of

mass spectra for known substances, the replication can be avioded, thereby the bioactivity of the evaluated compounds can be tested directly.[222]

The last screening program is target-oriented screening with the intention to determine the compounds that attack an established molecular target.[222] The cellular or molecular model of this target provides the significant platform for the drug development.[222] The attributes of target also play an essential role in this strategy, such as, without human homologue, wild distribution in bacteria, the location of the target in microbes and a low frequency of resistance.[222]

The biggest disadvantage of these traditional screening strategies is that thousands of compounds have been repeatedly detected.[229] It can be estimated that most of microbial metabolites have been harvested unless high-throughput screenings with larger screening libraries are implemented.[230]-[232]

Beside the traditional methods to discover the new antimicrobial agents, modern culture techniques and strategies are also accounting their own efforts for this field. Actinobacteria serve as the largest pool yielding enormous natural compounds, however only less than 1% of actinobacterial species have been successfully cultivated in laboratory so far whilst 99% of them still remain unculturable.[233] The main problem eager in need of solving is how the novel bacterial strains can be cultivated under conventional laboratory conditions.[222] Several new and revolutionary cultivation techniques have been pumped out so that a vast amount of initially uncultivable pathogens can be fostered.[222]

Microfluidic bioreactor cultivation belongs to one of these new methods. In this high-throughput cultivation system, up to 600000 pure soil-derived

Actinobacteria can be raised in microfluidic droplets per hour.[234] It can promote the slow growers under normal conditions yet inferior to fast-growing microbes and seed the possibility of new antibiotic compounds.[222] This idea aims to establish so far uncultured or unclassified actinomycetes and filamentous fungi in order to isolate new metabolites, has been used to found the company Naicons in 2006.[222] Owing to this, several new natural products have been found, for example, the protein synthesis inhibitor orthoformimycin, which owns a novel structure and possess a thoroughly rare mechanism of action, or an effective anti-inflammatory agent, the class III lanthipeptide NAI-112.[232]

On the other hand, *in situ* cultivation grows the microorganisms in a simulated environment. Kaeberlein *et al.* invented this technique via using diffusion chambers to imitate the marine environment in the case that two unknown microbes were isolated and termed as MSC1 and MSC2.[235] It was proved that MSC1 share 93% sequence similarity with its relative *Lewinella persica* by 16S rRNA gene sequencing.[235] Meanwhile, MSC2 is perhaps the relative of the species *Acrobacter nitrofigilis*, a common marine sediment microorganism related to nitrogen fixation.[235] It was further developed into a multichannel device assembled with hundreds of miniature diffusion chambers, each inoculated with a single environmental cell.[236] This device is termed as iChip (isolation chip), in which target bacterial strains rely on natural nutrients and are cultivated as single colonies in isolated microchambers so that the novel species can be fostered and easily transferred into pure culture.[236] Lassomycin and teixobactin are two novel antimicrobial agents obtained by this strategy.[237],[238] Lassomycin is isolated from *Lentzea kentuckyensis* and has excellent potency against Mycobacterium tuberculosis.[239] Teixobactin is obtained by previously

unculturable Gram-negative bacteria *Eleftheria terrae* and shows bioactivity against Gram-positive species, methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci and *Chlostridium difficile* while no resistant mutants of *S. aureus* and *M. tuberculosis* were detected.[240]

Co-culture technique provides another opportunity to access the potential drugs, in which bioactive compounds released from assistant bacteria impact the growth of other microorganisms so as to activate biosynthetic pathways as signals.[240] *Aspergillus*/*Streptomyces* co-cultivation has been done for exploration of new bioactive molecules.[241] Aspergicin and neoaspegicillic acid are the natural products produced by mixed-fermentation mycelia of marine-derived mangrove fungi.[242] Among them, Aspergicin reveals the antibacterial effects against many clinical pathogens and both Gram-positive and Gram-negative bacteria.[242]

Apart from sophisticated cultivation methodologies, Next-Generation Sequencing techniques are also able to detect novel complexes with potential therapeutic applications. A new bacterial taxon *Entotheonella* that co-inhabits the Red sea marine sponge *Theonella swinhoei* was identified by this single-cell and metagenomics-based method.[243] According to metagenomics analysis, more than 40 bioactive polyketides and altered peptides from seven structurally dissimilar genres were observed and released from the endosymbiont in the sponge.[243] This inspired example also enriched the novel reservoirs of microorganisms and their relative bioactive secondary metabolites.[240] Unexplored habitats, including desert, marine environment and endosymbiotic environment, are full of interesting molecules.[222] In the marine environment, salinity is perhaps the only parameter to be concerned in

association with microbial community composition and diversity, compared to temperature, pH value or other physical and chemical factors.[244] Marine actinomycetes are physiologically and phylogenetically distinguished from terrestrial species yet produce a vast amount of chemically diverse bioactive compounds as well.[245] Another abundant reservoir is the endophytes. The botanical diversity contributes to endophytic diversity in term of diverse plant life, tropic regions or temperate rainforests.[246] The species estimation of endophytic organisms could be up to 300000 due to the large number of unexplored plant species.[247] A review describing the natural products released from endophytic bacteria was stated, including ecomycins, pseudomycins, munumbicins and kakadumycins.[248] All of them possess antibiotic impact on different clinical pathogens. Therefore, it was affirmed again that bacterial endophytes own the ability of novel antimicrobials production.[248]

In addition, genome mining strategies have widespreaded in the last two decades,[249] where the biosynthetic gene clusters of natural products are initially investigated and determine whether these gene clusters are associated with the production of new antimicrobials.[250] For instance, the *Streptomyces nodosus* genome has 24 biosynthetic gene clusters for developing natural products, 18 of which are able to generate particular chemicals and their structurally relative compounds.[251] This convenient method brings a plentiful of beneficial advantages. The low cost or even no expend can be reached when the existing bioinformatics tools screen encoded genomes of potential molecules.[220] Adequate available DNA data nowadays are dedicated to this process as well.[220] Accessible sophisticated web-based tools, including anti-SMASH,[252]-[254] PRISM[255] or NaPDoS,[249] simplify the genome mining procedure, which can be propelled easily and reliably in natural product biosynthesis or

bioinformatics.[222] An intriguing outcome is that with more genetic discovery of bacteria, more diverse chemical compounds could be found than it was expected.[222] Moreover, the information of the biosynthetic gene clusters gives the chance to predict the chemical classes and structures of the encoded molecules.[222] This can direct a more targeted drug discovery technique (e.g. reactivity-guide isolation)[256] and peptide and glycogenomic approaches[257],[258] as well as the heterologous expression in an optimized expression host (also termed as activation of the silent gene cluster).[222] The comprehensive biosynthetic gene cluster databases, for instance, MiBIG (Minimum Information about a Biosynthetic Gene cluster) make the suggestion in elucidating the novel encoded compounds more conveniently.[259] To overcome the complexity between the gene clusters and bioactivity, a more cautious model named as target-directed genome mining was built, which detects potential resistance genes to yield more specific antibiotic compounds.[260] There are also some other methods strengthening *in silico* research, such as peptide and glycogenomics, which combine computational estimation with mass spectrometry[257],[258] or pattern-based genome mining, in which the vast amount of DNA sequence data is available to exploit.[261] The limitation of these computational genome mining processes is that they can only identify the biosynthetic gene clusters related to known biosynthetic mechanisms thereby the corresponding products are perhaps highly similar.[222] For the unknown biosynthetic pathway species, Identification of Natural compound Biosynthesis pathways by Exploiting Knowledge of Transcriptional regulation (INBEKT) approach was developed.[262] The signal-sensing regulators which promote or repress gene transcription by attaching to certain DNA motifs upstream of their target genes in response to environment factors, including nutrient starvation, oxidative stress and existence of competitive

microbial, were identified, therefore specific DNA-binding motifs and genome sequences were determined.[222],[262] The successful example of INBEKT is to identify the biosynthetic gene cluster of the zincophore ethylene diamine disuccinic acid ([*S*,*S*]-EDDS).[262] The zinc regulator Zur controls the production of (*S*,*S*)-EDDS in bacterial strain *Amycolatopsis japonicum* severely.[262] The determination of the Zur-binding motifs in the *A. japonicum* genome results in the finding of the (*S*,*S*)-EDDS biosynthetic genes eventually.[262]

The demand of new and fresh leads for antibiotic drugs is extremely high, since the antibiotic resistance has arisen immediately after the agents were introduced into the market. The barriers and some new perspectives for uncovering novel natural products can be summarized as four points. First of all, in present, there are very few antibiotic targets with a limited range of mechanisms.[23] Meanwhile, the outcomes of genomic tools have not reached the ideal expectation.[23] Unconventional targets and discovery platforms could be an alternative solution since great efforts have been invested in conventional ones however replication seems to be a powerful disadvantage.[23] Secondly, the mechanisms of action of existing antimicrobial agents are too complicated to apply in modern drug discovery.[23] A comprehensive understanding of these mechanisms should be done in the first place. Besides, the physical and chemical properties of current antibiotics bring the burden to the conventional collections of compounds and medicinal-chemistry methods.[23] In particular, physical characteristics of chemicals which can overcome the problem in permeability of the Gram-negative species deserve great efforts.[23] The last point is to reconsider the determinants for identification of novel compounds.[23] Waksman platform has been employed in this field since invented and reached a superior result meanwhile restricted the protocol where numerous targets, for

instance virulence factors and in terms of metabolism, are not seized in this traditional MIC approach.[23] Besides, further ideas can be taken in consideration as well. Some compounds that only inhibit the pathogenic action instead of slaughtering the bacteria could avoid acquisition of mutations.[222] Another strategy is to combine different sorts antimicrobials based on clinical experiments and patient records.[23] It displays compelling impact on medical treatments nowadays, such as the combined usage of trimethoprim and sulfamethoxazole can result in the synergistic influence in folate metabolism or cooperation between amoxicillin and clavulanic acid, former is a β-lactam antibiotic and later is an inhibitor of β-lactamase drug resistance enzymes, can accomplish synergy and restrain a broad spectrum of microbes.[23]

On the other hand, millions of synthetic chemicals have been produced for various purposes. To some extent, this can also enlarge the possibility for seeking new medicines. The synthetic fluoroquinolones can be exemplified as one of the most successful antibacterial agents for several decades.[23] However, criteria like the Lipinski rule of five restricted the imagination firmly. In this rule, a potential orally active drug should have less than 5 hydrogen bond donors and less than 10 hydrogon bond acceptors, while its molecular mass cannot beyond 500 daltons.[263],[264] Besides, the octanol-water partition coefficient log *P* should be controlled under 5.[265] Yet, obeying to this rule couldnot simply guarantee the pharmacological activity of the candidate drugs. Therefore, for the discovery of novel oral medications, synthetic strategies focus mainly on the compounds optimized for human biology. As natural products and their derivatives, most of existing antibiotics cannot adapt those parameters.[266] Meanwhile, the synthetic molecules could not accomplish the biological functions as the natural antimicrobial agents, such as penetration of the cell membranes.[23] Thus, the situation

is placed in a dilemma that the compounds from screening natural-product libraries are bioactive yet formerly known, whilst the outcome of synthetic libraries could yield potent ligands of biochemical targets with impoverished bioactivity.[23]

Despite the necessity for new generations of antibiotics, the development of fresh leads particularly in those that capture deadly multidrug-resistant Gram-negative bacteria, is however pulled back due to high risk and low profit to the pharmaceutical and biotechnology firms.[267] The reasons for antibiotics transforming from magic bullets into an financially unattractive object in less than one century can be depicted as below. First, compared to other drug classes, antimicrobials show less profit due to national conservation, an established generics market and short-term treatment.[268],[269] Second, the uncertainty of the regulatory requirement for market approval in US and European Union adds more barriers to be overcome.[270] Third, the investment in production of new drugs is shifted to other more profitable areas by many pharmaceutical companies.[267],[271] Therefore, the academic and basic research should contribute more effort in propelling the development of novel antibiotics than previously.

1.5 Fungal volatile organic compounds

A variety of volatile organic compounds (VOCs) with low molecular weight are produced by individual fungal species via primary and secondary metabolism pathways.[272] These compounds are easily evaporated into the gas phase at normal atmospheric temperature and pressure.[273] In general, they have a special donor and are low to medium soluble in water.[274] For example, 1-octen-3-ol (also termed as muchroom alchol) was identified as main aroma compound from distinctive mushromm species, including *Agaricus bisporus*,[275],[276] and

mold genera like *Aspergillus* and *Penicillium*.[277] Due to their certain properties, fungal VOCs are frequently considered as very practical and useful chemicals for agriculture, food and flavor industry.[273] Along with the development of the modern analytical techonology, more and more chemically different and biologically active fungal VOCs have been found, including alcohols, acids, aldehydes, esters, ketones, thiols, aromatics, terpenes, and so on.[273] Besides, they are also a rich natural source for the investigation of their biotechnological potential as mediators between fungi and plants, arthropods, bacteria, and other fungi.[278] However, in comparison to the study about the VOCs with bacterial,[279] plant, or synthetic origin, far less attention was paid to the VOCs of fungal origin.[273] Therefore, my research interest in the topic of the investigation of the secondary metabolites from fungi was raised and fulfilled by studying the fungal representatives of *Geniculosporium* and Xylariacea, in terms of VOCs collection, detection, characterization and bioactivity evaluation.

1.5.1 *Geniculosporium*

Endophytic fungi can be found in almost all plants, including trees, grasses, algae, mosses and herbaceous plants.[280] Most of them arise from Ascomycetes and Fungi imperfecti.[281] In ordinary circumstances, they rely on the host plant for living in the absence of apparent diseases.[281] When the host feels the threat from environmental stress, endophytes might turn into pathogens, thereby the delicate equilibrium between host and endophytes might be broken and chemical factors impact a lot in this balance.[282] For instance, herbicidal natural products are released by the fungi and antifungal compounds are biosynthesized by the host plant.[282]

Endophytic fungus *Geniculosporium* sp. (strain number 2121) with callus of its host *Teucrium scorodonia* is able to produce the bioactive diterpene geniculol (**29**) and the acknowledged fungal metabolite cytochalasin F (**30**), both of which exhibit algacidal properties (Figure 7).[280] In addition, cytochalasin F displays inhibition of photosynthesis and has an ecological function in the plant-symbiont interaction.[280] In previous study, several volatile lactones (**31**-**33**) have been detected from *Geniculosporium* sp. 9910 , which was isolated from the rockrose *Cistus monspeliensis*, also termed as Montpelier cistus.[283] These lactons were collected from agar-plate cultures by usage of a closed-loop stripping apparatus.[283] Unambiguous compound characterisation was done by GC-MS and verified by synthesis of reference compounds.[283] Pogostol (**34**), a sesquiterpene was found in the same fungal strain and its putative biosynthesis was investigated by feeding experiments with six ^{13}C-labelled isotopomers of mevalonolactone in cooperation with ^{13}C-NMR analysis.[284] It is noted that marine microorganisms can obtain different natural products compared to their terrestrial relatives. For example, fourteen new tricyclic sesquiterpenes (**35**-**48**) with the botryane skeleton and four known cytochalasins (**49**-**52**) were isolated from *Geniculosporium* sp. (internal strain number 6580) associated with the red alga *Polysiphonia* sp. from the Baltic Sea at Ahrenshoop, Germany.[285] The novel botryane compounds (**35**-**48**) have some inhibitory impact in microbes *Chlorella fusca*, *Bacillus megaterium* and *Microbotryum violaceum*.[285]

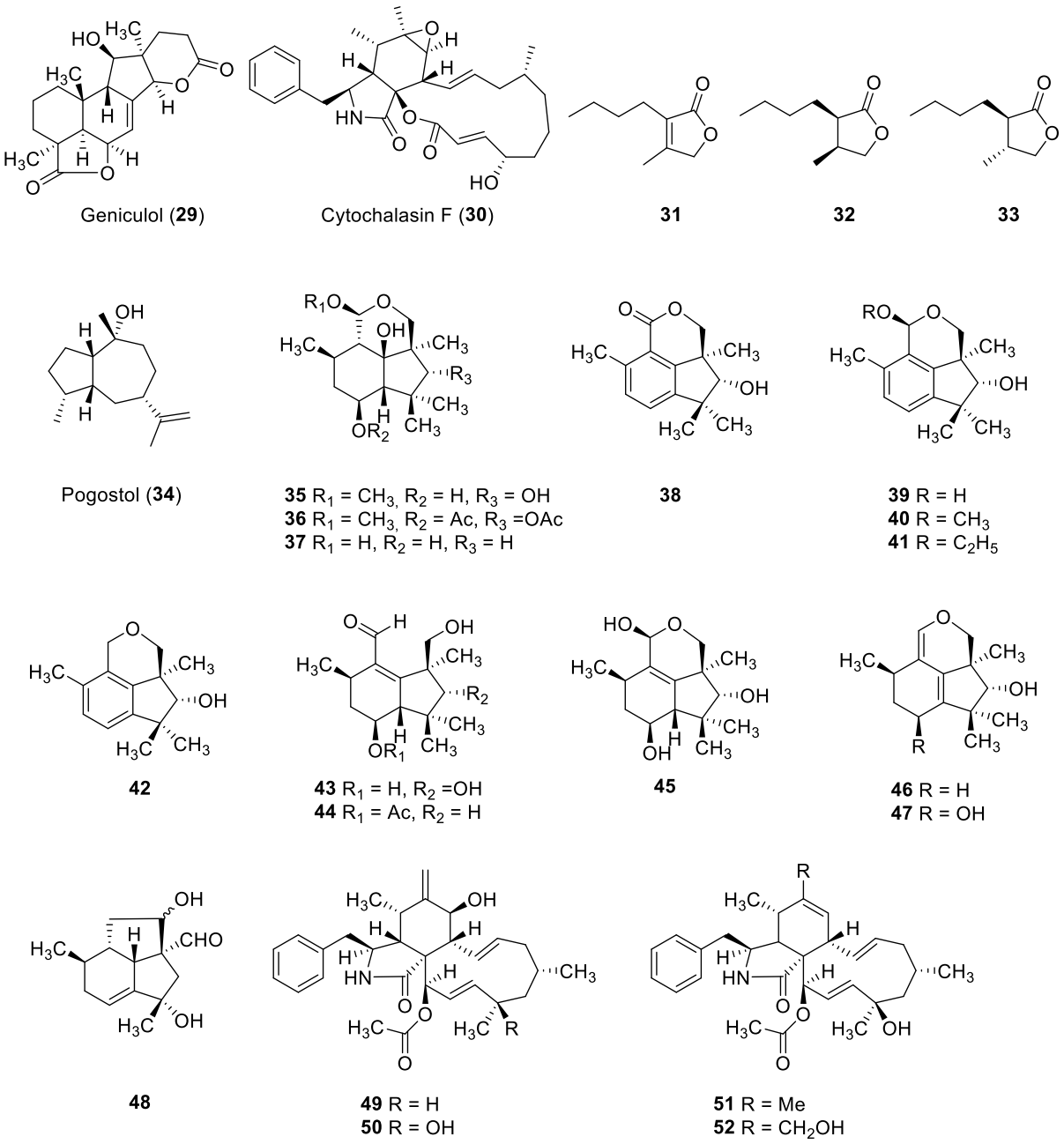


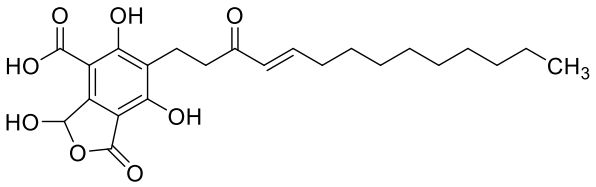
Figure 7 Secondary metabolites from Endophytic fungus *Geniculosporium* sp.

1.5.2 Xylariaceae

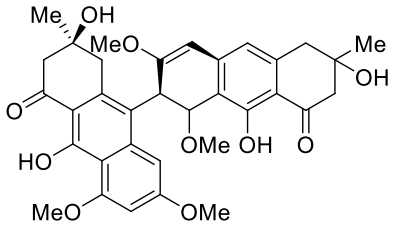
Xylariaceae is one of the largest families of the Xylariales (Ascomycota) comprising over 85 genera and far more than 1340 species.[286] It is assorted into two main groups (Hypoxyloideae and Xylarioideae) essentially based on an abundance of distinguishing features, including anamorphic type, form of stroma, extractable stromatal pigment and

other secondary metabolites.[287]-[290] Xylarioideae constitutes the largest diverse type genus of Xylariaceae.[290] *Xylaria* and its allies share mostly *Geniculosporium*-like anamorphs.[290] It owns characteristic stromata as a clavate to stipitate or conspicuously constricted habit.[290] In some wood-inhabiting *Xylaria* spp. (*X. polymorpha*, *X. hypoxylon*), it can be easily identified due to its extremely large size in every mushroom field.[290] Some other *Xylaria* spp. can only generate inconspicuous stromata in tropical fruits, insect nests and so on.[290] Initially, the hydroxyphthalide derivative xylaral (**53**) was the only compound isolated from *X. polymorpha*, which can obtain violet colour in aqueous ammonia (Figure 8).[291] Later, phlegmacin (**54**) and other emodin type stromatal pigments have been investigated from *Xylaria euglossa*.[292] The established metabolites exist also in the Basidiomycetes of the genus *Dermocybe*, while the emodin pigments can be found in many ascomycetes.[293] Hypoxyloideae as the second major subgroup of Xylariaceae is comprised of the genus *Hypoxylon* and its immediate allies, such as *Biscogniauxia*, *Camillea*, *Daldinia*, *Entonaeme* and small tropical taxa.[290] They all show up a *Nodulisporium*-like anamorph and can be further separated into the *Biscogniausxia*/*Camillea* complex and *Hypoxylon* together with its immediate allies according to appearance of stromatal pigments.[290] Dihydroisocoumarins, like formylmellein (**55**) was described in culture of *Biscogniauxia marginata* and also later found in other species of *Biscogniauxia*.[294]-[297] Characteristic pigments deposited in granules directly between the perithecia and under the stromatal surface, exist in most of *Hypoxylon* species.[290] Mitorubrins (**56**-**58**) were the first chemical compounds of these pigments detected from *H. fragiforme*.[298] Besides, they might also release chemically related azaphilones, like hypomiltin (**59**), the rubiginosins and entonaemins (**60**-**63**), and the fatty acids, rubiginosic acid (**64**).[299]-[301] Endophytic Xylariaceae same as

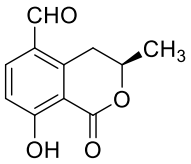
other endophytic fungi, have been explored for mass production of volatile organic compounds. Up to date, there are only a few studies on the analysis of the produced volatiles from this family. For instance, 1,8-cineole was discovered from a series of VOCs emitted from *Hypoxylon sp.* and related anamorph *Nodulisporim sp*.,[302]-[304] the comparison of emitted volatiles between *Daldinia concentria* and *Daldinia hawksworthii*,[305] and identification of 27 VOCs from *D. cf. concentrica* by gas chromatography-mass spectrometry analysis.[306]



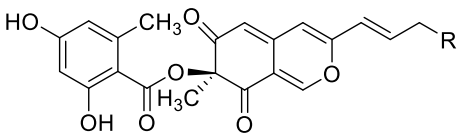
Xylaral (**53**)



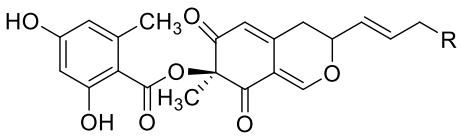
Phlegmaicin (**54**)



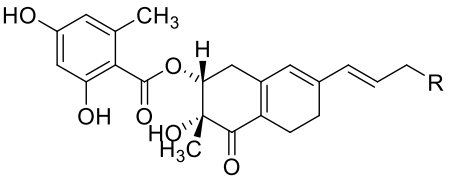
5-Formylmellein (**55**)



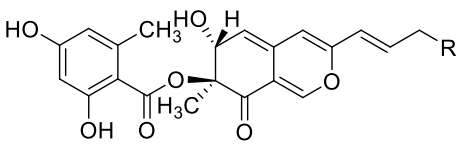
Mitorubrin (**56**) R = H
Mitorubrinol (**57**) R = OH
Mitorubrinol acetate (**58**) R = $OOCCH_3$



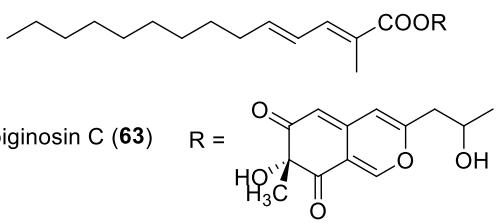
Hypomiltin (**59**) R = $OOCCH_3$



Entanaemin A (**60**) R = OH
Rubiginosin A (**61**) R = $OOCCH_3$



Rubiginosin B (**62**) R = $OOCCH_3$



Rubiginosin C (**63**)

Rubiginosic acid (**64**) R = H

Figure 8 Secondary metabolites from Endophytic fungus *Xylariacea*.

1.6 Linezolid

Except obtaining the immense inspiration from nature, modification of chemical skeletons of existing antibiotics could be another approach to develop novel potential candidates. Therefore, a completely different project regarding to alternation of linezolid, the first synthetic oxazolidininone antibacterial agent, was present in this thesis. Production of modified linezolid derivatives was made and biological activity was reported in correlation with their chemical structure.

In general, the discovery of linezolid can be divided into several phases. The DuPont pharmaceutical initially reported two new antimicrobial compounds in 1987, DuP-105 and DuP721.[307] These compounds were the first representatives of oxazolidininone antibiotic family and stemmed from a series of racemic 5-halomethyl-3-phenyl-2-oxazolidinones with properties for treatment of various plant diseases, for instance compound **65** and its chemically modified molecule **66**.[308] Compound **66** displays *in vitro* activity and *in vivo* efficacy against several Gram-positive and Gram-negative pathogens.[309] The exploration to **66** elucidated the absolute configuration of the oxazolidinones, particularly at C-5, which exhibits the essential antibacterial activity (Figure 9) and eventually inspired the production of its descendants DuP-721 and DuP-105.[310]

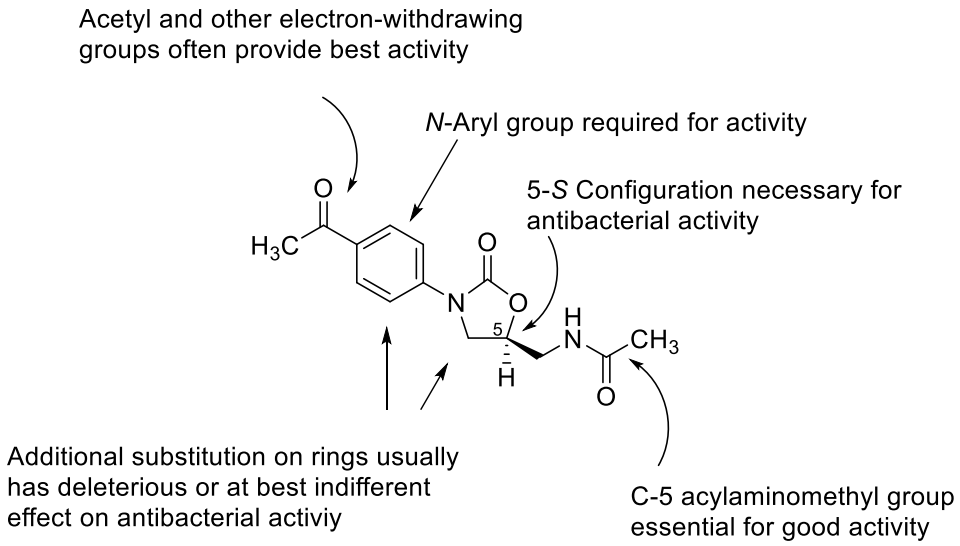


Figure 9 Early structure-activity relationships of oxazolidinones examined at DuPont.[311]

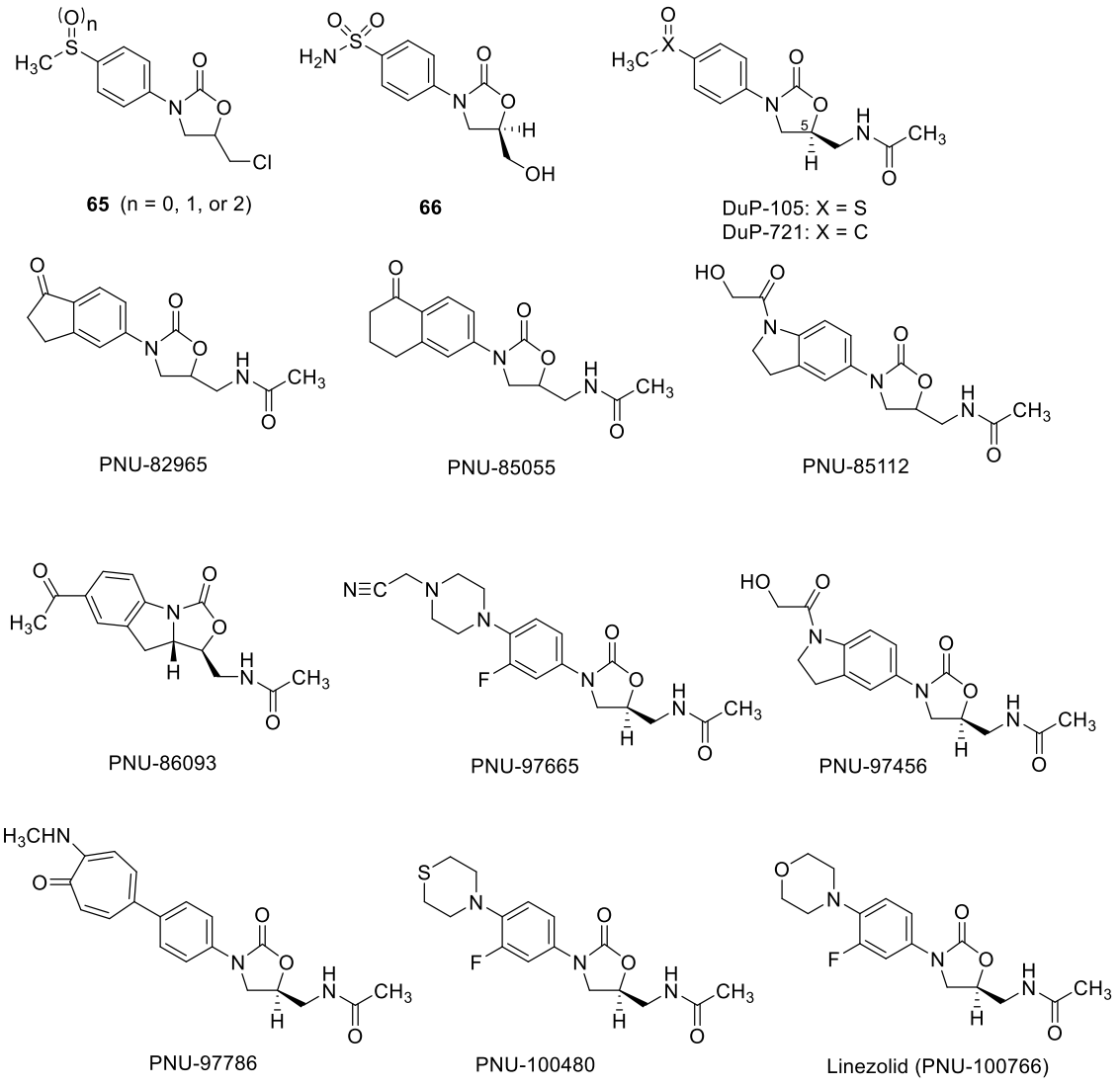
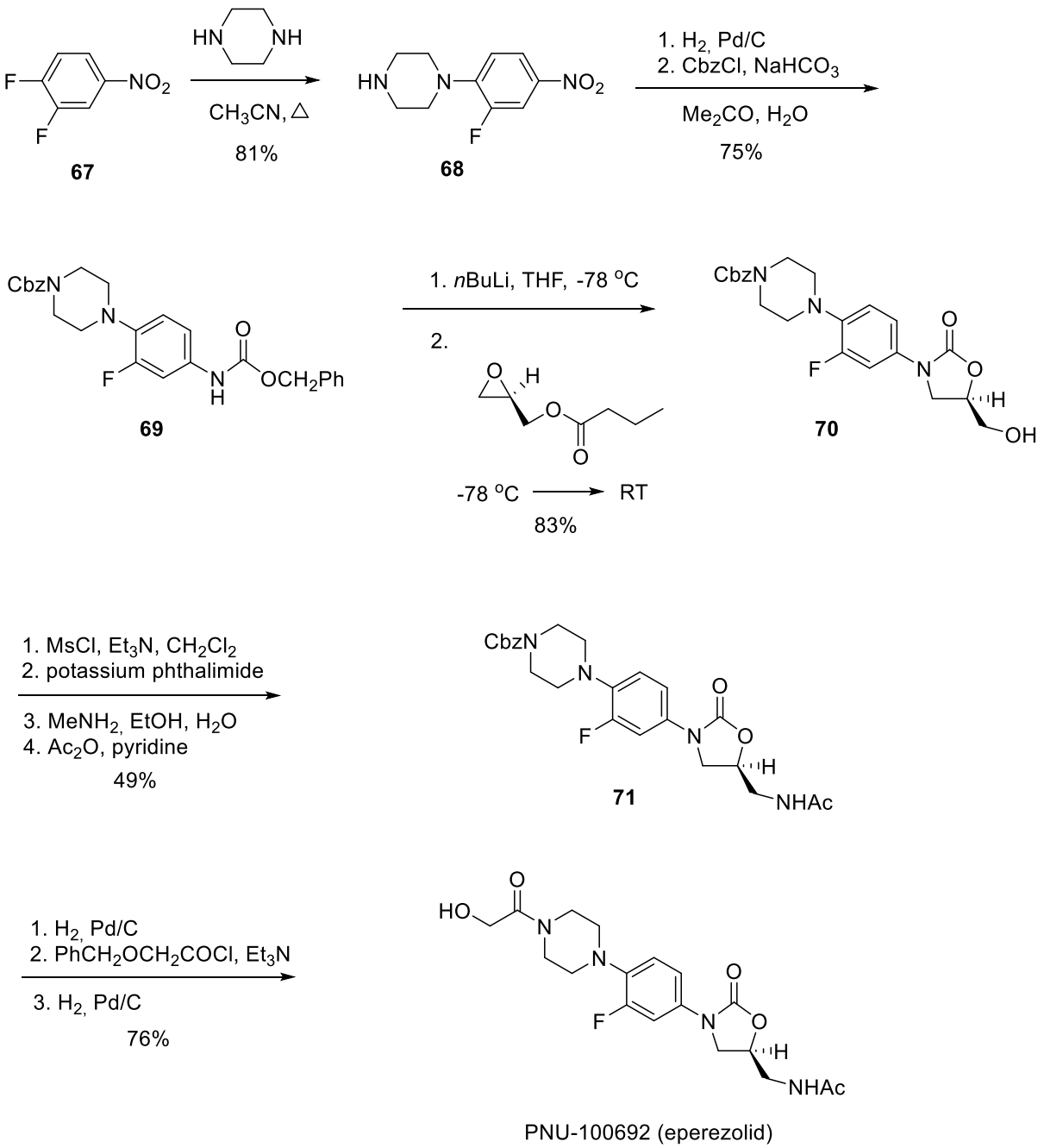


Figure 10 Development of oxazolidinone antibacterial agents.[311]

Later the assigment was passed to Pharmacia company, in which two analogues PNU-82965 and PNU-85055 containing bicyclic ring system (indanone and tetralone) instead of the common 4-substituted phenyl ring were accomplished and tested.[312],[313] To be noted, only the *S* enantiomer was found to be antibacterial active, since the racemic material demonstrated exclusively half the potency of the pure enantiomer.[310] The safety evaluation of racemic DuP-721 and PNU-82965 was conducted to declaim that PNU-82965 indicated less aggressive lethal effect than racemic DuP-721 in the clinical evidences of the treated rats.[314] This also proved that a structure-toxicity relationship remains in this family.[311] Another racemic indoline congener PNU-85112 was subsequently generated and showed an excellent safety profile in the toxicity study while impacted *in vitro* activity and *in vivo* efficacy similar as racemic DuP-721.[315] Afterwards, even a tricycle fused racemate PNU-86093 was designated and synthesized to enhance the oxazolidinone pharmacophore.[316] Unfortunately, it displayed in vitro antibacterial activity merely lower than racemic DuP-721.[311]

By the end of 1992, three major types of oxazolidinone analogues were discovered, including piperazinylphenyloxazolidinones (e.g. PNU-97665),[317] indolinyloxazolidinones (e.g. PNU-97456)[318] and the trophonylphenyloxazolidinones (e.g. PNU-96686).[315] The indolines displayed a superior safety profile yet poor antibacterial activity.[311] However, the troponyl compounds with excellent antibacterial activity revealed impoverished water solubility and poor pharmacokinetic characteristics.[311] Unlike the others, piperazine series retained not only outstanding *in vitro* and *in vivo* activity but also in possession of an acceptable safety profile, acceptable water solubility and remarkable pharmacokinetic parameters.[311] Besides, these analogues were also the easiest obtainable compounds via synthesis.[311] After screening several

candidates in this group, it was observed that one or two fluorine atoms flanking the *para* piperazine group indicated considerable potentiating impact on the antibacterial activity and hydroxyacetyl moiety was deemed to be the optimal nitrogen substituent.[311] In consistence with this perspective, the monofluorophenyl molecule PNU-100594 with the optimal level in antibacterial activity, pharmacokinetics, water solubility and other pertinent properties was eventually generated.[319] An early synthesis report is depicted in Scheme 6. It starts with 3,4-difluoronitrobenzene (**67**) being treated with excess piperazine via nucleophilic aromatic displacement to afford the *p*-substituted nitrobenzene **68**, selectively. Reduction of **68** and subsequent attachment with a carbobenzoxy (CBZ) activating group reached compound **69**. Deprotonation with *n*-BuLi and later stirring together with (*R*)-glycidyl butyrate obtained compound **70** with a good yield (83%). Mesylation of **70** and reaction with potassium phthalimide give an intermediate. After that, cleavage of the phthalimide group and replacement with aqueous $MeNH_2$ was followed by the treatment of Ac_2O and pyridine to have **71**. It was deproteted into amine and acylated with (benzyloxy)acetyl chloride. The last step was the benzylic hydrogenolytic cleavage to yield PNU-100692 (eperezoild) as final product.[319]



Scheme 6 Total synthesis of eperezolid (PNU-100592). Cbz = benzyloxycarbonyl, Ms = methanesulfonyl.[319]

Replacement of the piperazine ring led to the discovery of antimycobacterial thiomorpholine derivative PUN-100480[320] and morpholine analogue PNU-100766,[319] termed as linezolid afterwards. The adjusted structure-activity relationships of oxazolidinone based on earlier DuPont work were exposed by Pharmacia and summarized in Figure 11.[311] Two interesting points could be conducted in this

investigation. An appropriate electron-donating amino substituent on the phenyl ring can contribute to brilliant antibacterial activity and a good safety profile.[305] One or two fluorine atoms flanking the morpholine or piperazine ring could enhance antibacterial activity as well.[311] A brief large-scale production of linezolid was demonstrated in Scheme 7.[321] A nucleophilic aromatic displacement of 3,4-difluoronitrobenzene (**67**) with excess morphine was followed by reduction to yield amine **72**. Then, the attachment with a carbobenzoxy (CBZ) activating group was accomphlished and obtained **73**. *N*-alkylation of amide was done by treatment of (*S*)-(+)-3-chloro-1,2-propanediol in order to introduce 5-*S* stereochemical center, subsequently deprotonated and oxazolidinone ring closure by potassium *tert*-butoxide and LDA to reach compound **74**. Afterwards, hydroxyl group was protected by 4-nitrobenzensulfonyl chloride to afford **75**. Later, it was converted by ammonia into the corresponding amine **76**. Finally, acetylation gave linezolide (PNU-100766). [321]

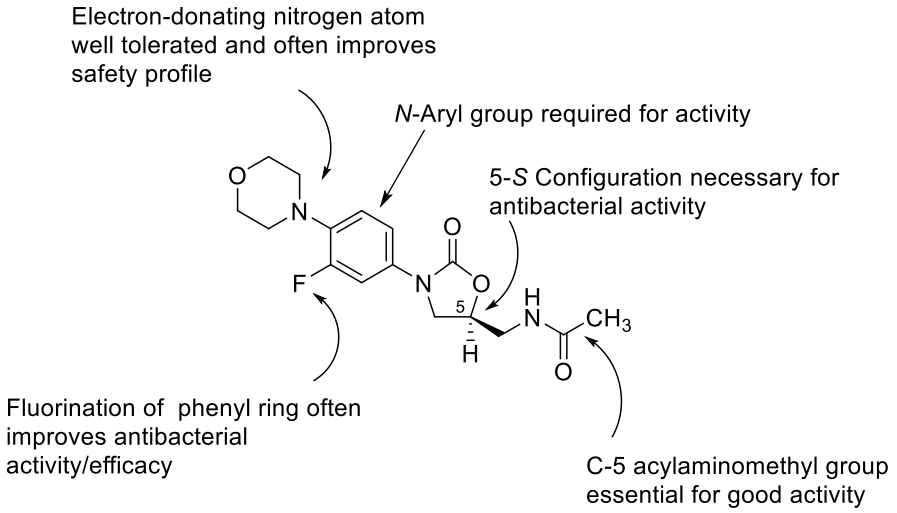
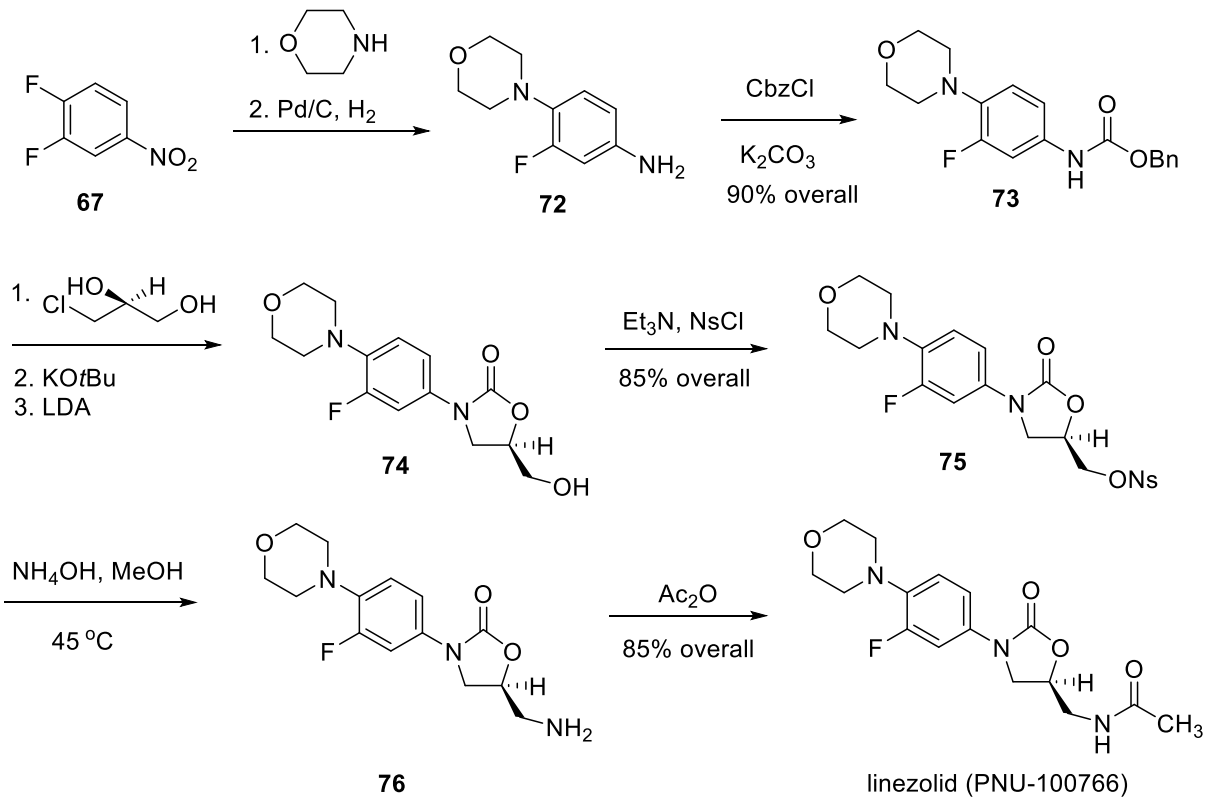


Figure 11 Revised structure-activity relationships evaluated at Pharmacia.[311]



Scheme 7 Total synthesis of linezolid (PNU-100766). LDA = lithium diisopropylamide, Ns = *meta*-nitrophenylsulphonyl.[321]

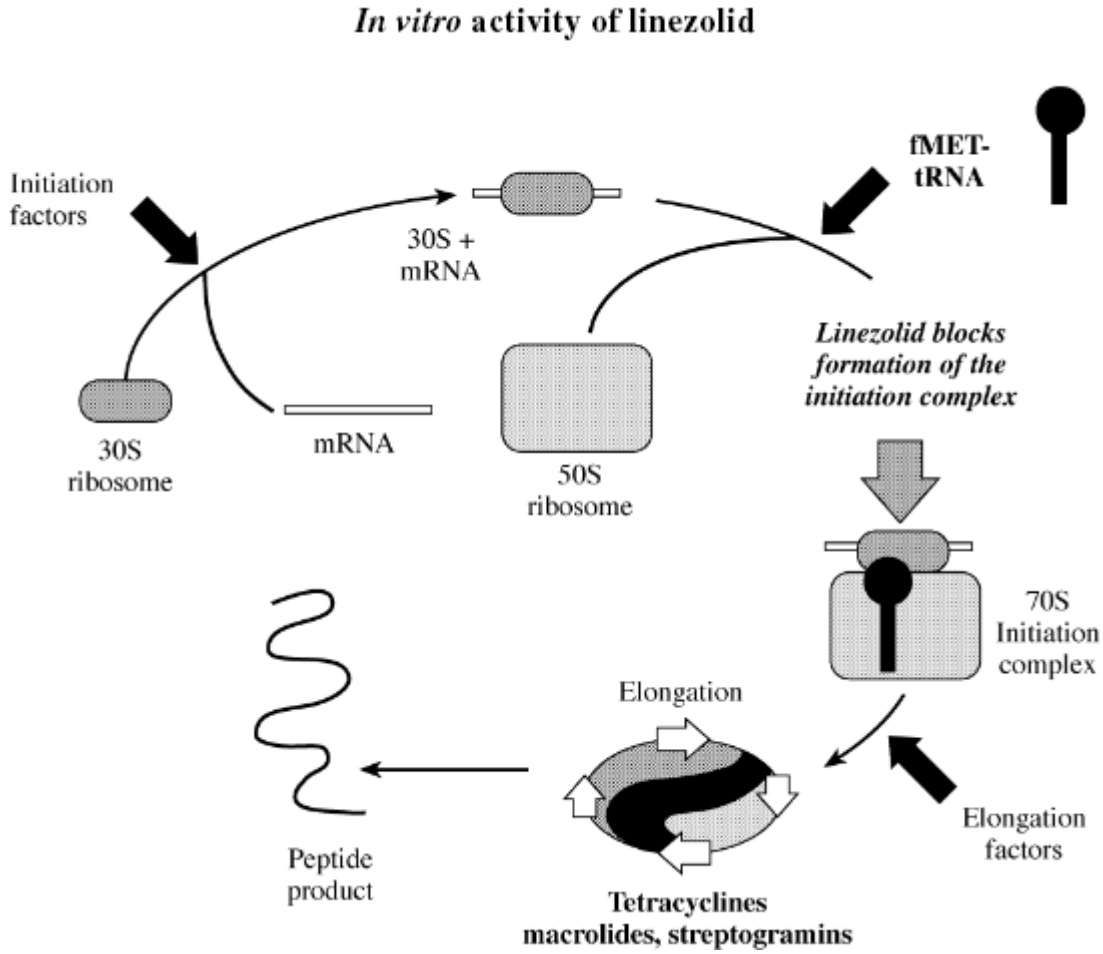


Figure 12 Mechanism of antibacterial action of the linezolid.[322]

Oxazolidinones share a unique characteristic mechanism of action which avoids them being targeted by existing resistance mechanism in bacteria (Figure 12). Oxazolidinones bind to the 50S ribosomal subunit, preventing it from complexing with the 30S subunit, mRNA, initiation factors and formylmethionyl-tRNA.[323],[324] More specifically, Linezolid binds to the A site pocket at the peptidyltransferase center of the ribosome overlapping the aminoacyl moiety of an A-site bound tRNA.[325] This induces a newly distinct conformation of the universally conserved 23S rRNA nucleotides and consequently results in a nonproductive state for peptide bond formation, blocks assembly of a functional initiation complex for protein synthesis and impairs translation of the mRNA.[322],[325] It differs from other protein synthesis inhibitors,

exemplified as chloramphenicol, macrolides, lincosamides and tetracyclines, which tolerate mRNA translation afterwards, constraining peptide elongation.[322]

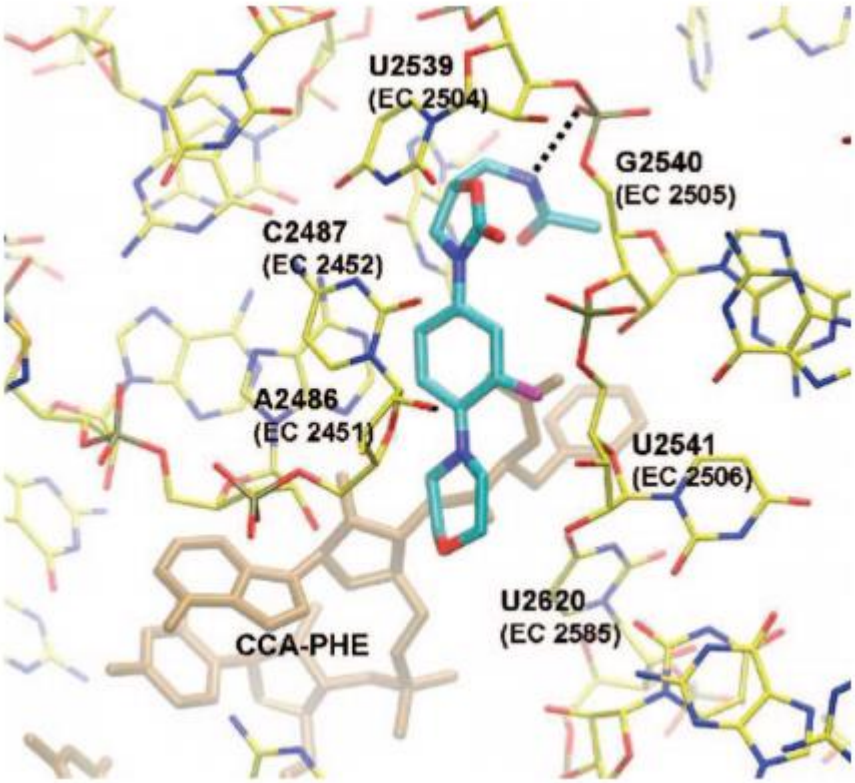


Figure 13 Overview of linezolid (cyan) and CCA-PHE (gold) binding to *Haloarcula marismortui* 50S ribosomal subunit. The acetamide arm hydrogen bonding to G2540 is indicated.[326]

Two crystal structures of the oxazolidinone antibiotic linezolid bounding to the *Deinococcus radiodurans* and *Haloarcula marismortui* 50S ribosomal subunits were determined respectively and offer a definitive visualization to understanding the mechanism of action.[325],[326] For instance, Figure 13 demonstrates the complex of linezolid and CCA-*N* acetylphenylalanine (CCA-Phe) binding to *Haloarcula marismortui* 50S ribosomal subunit. In it, hydrogen bonding and hydrophobic packing interactions are frequently utilized by linezolid in terms of connecting an all-RNA pocket.[326] The oxazolidinone ring representing the

pharmacokinetic property is stacked against base moiety of U2539 and results in van der Waals interactions.[326] A hydrogen bond between the acetamide NH and the phosphate group of G2540 could be observed as well.[326] The fluorophenyl moiety of linezolid is located in a heteroaromatic crevice comprised of residues A2486 and C2487 in the peptidyltransferase center, named also as A-site cleft.[326] This conformation has shown hydrophobic functionality in other antibiotic inhibitors.[327] However, the morpholine ring does not exhibit any significant interactions with the ribosome, so that distinct functional groups can replace it in the absence of significant loss in activity.[311] The elucidated crystal structures of linezolid bound to the 50S subunits of prokaryotic ribosomes provide a structural basis for the discovery of next-generation oxazolidinones active against emerging drug-resistant clinical strains, including linezolid-resistant strains.[328]

In accordance with the crystal structure of linezolid targeting *Haloarcula marismortui* 50S ribosomal subunit, *in silico* prediction for new and promising lead compounds has been conducted and several novel distinct oxazolidinone antibacterial agents were given from it.[329] Reliable force field simulations (the AMBER and the OPLS-AA force fields), first principle calculations and accessible experimental data have all contributed to rebuilding the conformational space of linezolid in association with the isolated and the ribosomal bound state.[329] Initially, an all-atom bacterial ribosomal model with nearly 1600 atoms was constructed and examined.[329] Stochastic Monte Carlo (MC) method was employed to scan the conformational space with 30 different ribosomal/oxazolidinone complexes.[329] Afterwards, their enthaplic penalties were calculated respectively according to Equation 1, where $\Delta\Delta Eb$ represents the binding enthalpy relative to linezolid and the rest stand for the enthalpies of the complex between the ribosome and

specific linezolid analog ($E_{\text{ribo-guest}}$), the complex between the ribosome and linezolid ($E_{\text{ribo-lzd}}$), the solvated linezolid analog (E_{guest}) and the solvated linezolid (E_{lzd}).[329]

$$\Delta\Delta Eb = (E_{\text{ribo-guest}} - E_{\text{ribo-lzd}}) - (E_{\text{guest}} - E_{\text{lzd}}) \quad (1)$$

Also, the mechanical strengths of the relevant hydrogen bonds were evaluated, including relaxed force constants and compliance constants.[329] Eight linezolid analogues with the experimentally known MIC (Minimal Inhibition Concentration) values were applied for crosschecking the robustness of the fresh-made model, by comparison to their calculated relative enthalpic binding energies, partially demonstrated in Table 1.[329] Based on this protocol, 22 new linezolid derivatives (**80**-**101**) in Figure 14 were chosen merely by chemical intuition and their ribosome affinities were valued by the enthalpic penalties.[329] These potential antibiotic compounds enrich the arsenal and give valuable inspirations toward the rational development of the next generation of oxazolidinone antibiotics.

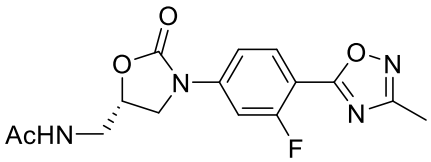
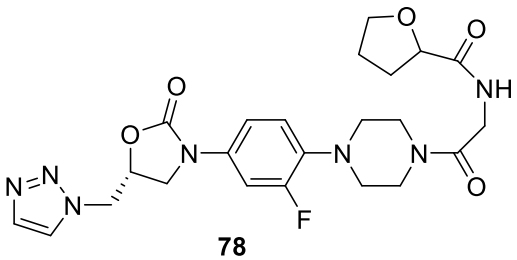
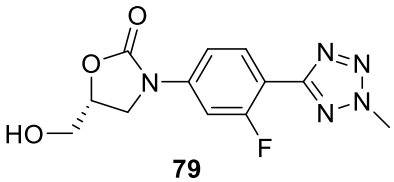
Guest	ΔΔ*Eb* [kJ/mol]	MIC value[a]
 **77**	-15.6	2
 **78**	-5.2	8
 **79**	-27.9	0.5
Linezolid	0.0	1-2

Table 1 Comparison between computed relative enthalpic binding energies and the experimentally known MIC (Minimal Inhibition Concentration). [a]Minimal Ihibition Concentration values detailed in mg/L for seletcted methicillin-resistant Staphylococcus aureus bacterial strains (MRSA).[329]

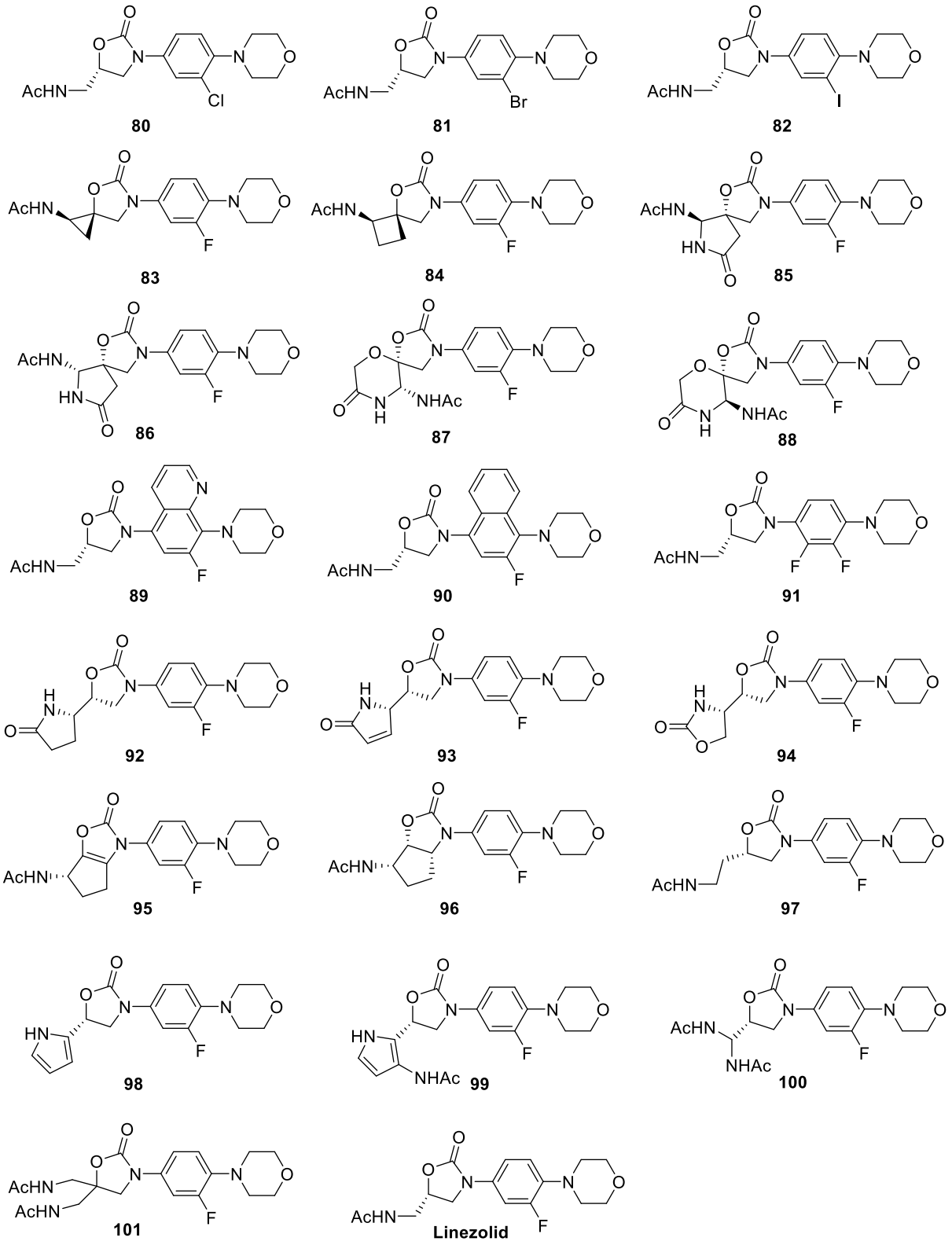


Figure 14 Predicted new linezolid derivatives.[329]

Among these new compounds, molecule **92** owns the lowest relative enthalpic energy in binding affinity resulted from the theoretical

calculation. According to the computer simulation (Figure 15), two hydrogen bonds between oxazolidinone **92** and ribosomal 50S subunit emerged. One occurs between the NH in oxazolidinone and A2538, whilst another binding indicates between the oxazolidinone-ester-type oxygen and the G2012 guanine NH group.[329] This assumed that compound **92** could be an outstanding candidate with excellent antibacterial properties. Therefore, a chemical synthetic approach was planned to reach this target. Its biological evaluation should be done for searching the antimicrobial properties.

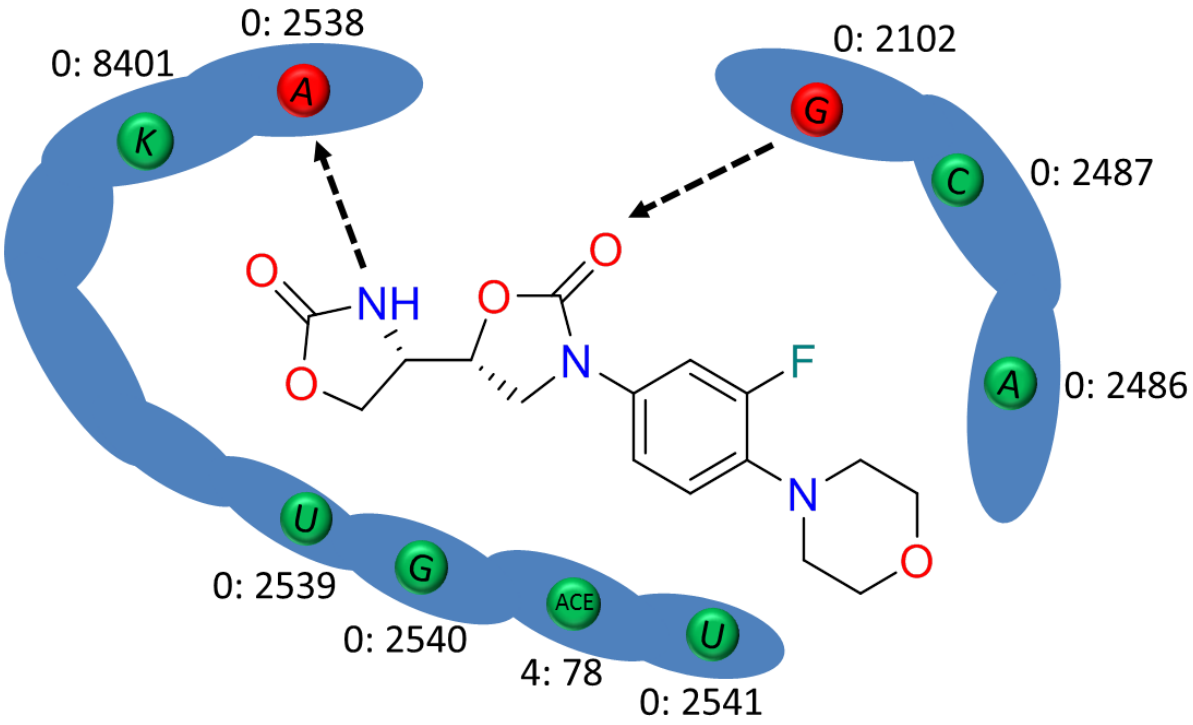


Figure 15 Illustration of the conformation and interaction between oxazolidinone **92** and ribosomal 50S subunit (blue area). Red colour balls stand for amino acid residues binding to compound **92** via the hydrogen bonds depicted as black dashed arrow lines, while green colour balls represent amino acid residues surrounding the compound.[329]

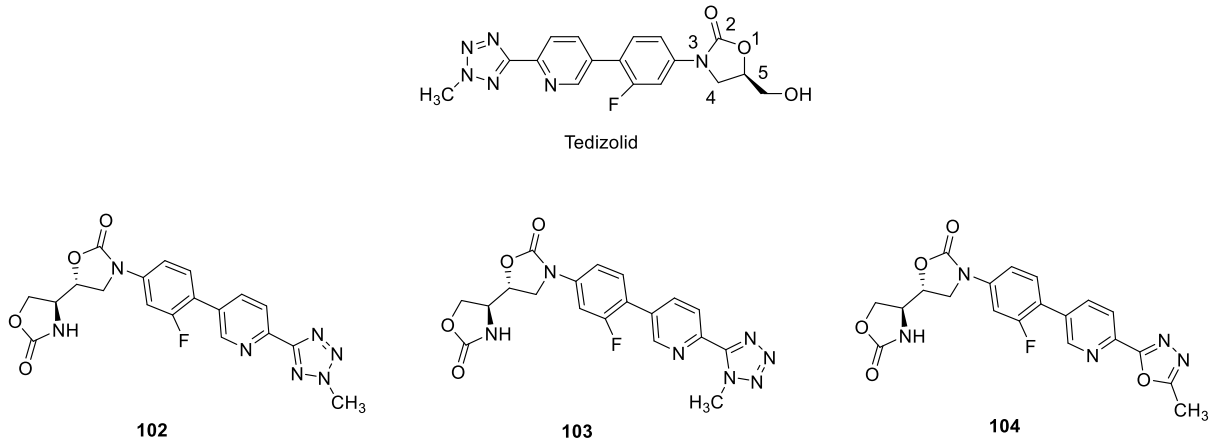


Figure 16 Tedizolid and its derivatives (**102**-**104**).

In the study of oxazolidinone antibacterials, tedizolid is considered as another interesting relative topic. Its phosphorylated compounds service as an inactive prodrug and would be turned into tedizolid rapidly and extensively after oral or intravenous administration to treat acute bacterial skin and skin structure infections (ABSSSI).[330] It also exhibits excellent activity against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and has been approved for usage by FDA in 2014.[331] Tedizolid shares similar structures as linezolid or other oxazolidinone compounds, yet small modifications in the molecule resulted in four- to eightfold more potency *in vivo* than linezolid against a variety of bacterial pathogens.[331] For example, a hydroxymethyl group at the C-5 position instead of the acetamide group in linezolid can display potency against linezolid-resistant bacterial strains with the *cfr* gene, which decreases oxazolidinone susceptibility in terms of shortage of certain ribosomal mutations.[331] The optimization of pyridine and tetrazole rings reinforces the additional binding interactions with upper region of peptidyltransferase center (PTC) of the 50S ribosomal subunit.[332] Its derivatives **102**-**104** (Figure 16) with similar novel two oxazolidinone rings as in compound **92** were considered as synthetic targets for this thesis as well.

2. Results and Discussion

2.1 Analysis of volatiles emitted by fungus *Geniculosporium*

The agar plate cultures of the endophytic fungal genus *Geniculosporium* sp. 9910 released the volatile compounds, which were collected on a charcoal filter through the closed-loop stripping apparatus (CLSA) headspace technique.[333] After a collection time of 24 hours, the adsorbed filter was extracted with analytically pure dichloromethane and the extracts were analyzed by GC-MS. The analytical results were obtained three times to prove its reproducibility, and a representative chromatogram is demonstrated in Figure 17. The headspace extracts were composed of major compounds *cis*- and *trans*-lactones (**32** and **33**), and 2-butyl-methylbut-2-enolide (**31**). They were reported previously from the same fungal species,[283] along with minor metabolites **105** and **106**.

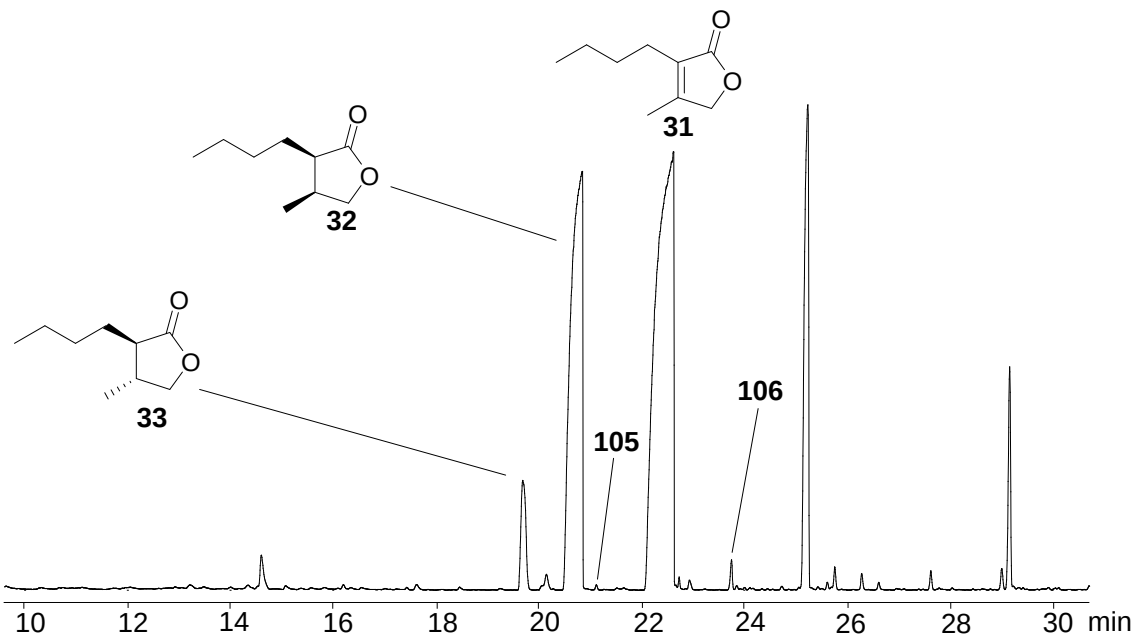


Figure 17 Total ion chromatogram of a representative headspace extract from *Geniculosporium* sp. 9910.[283]

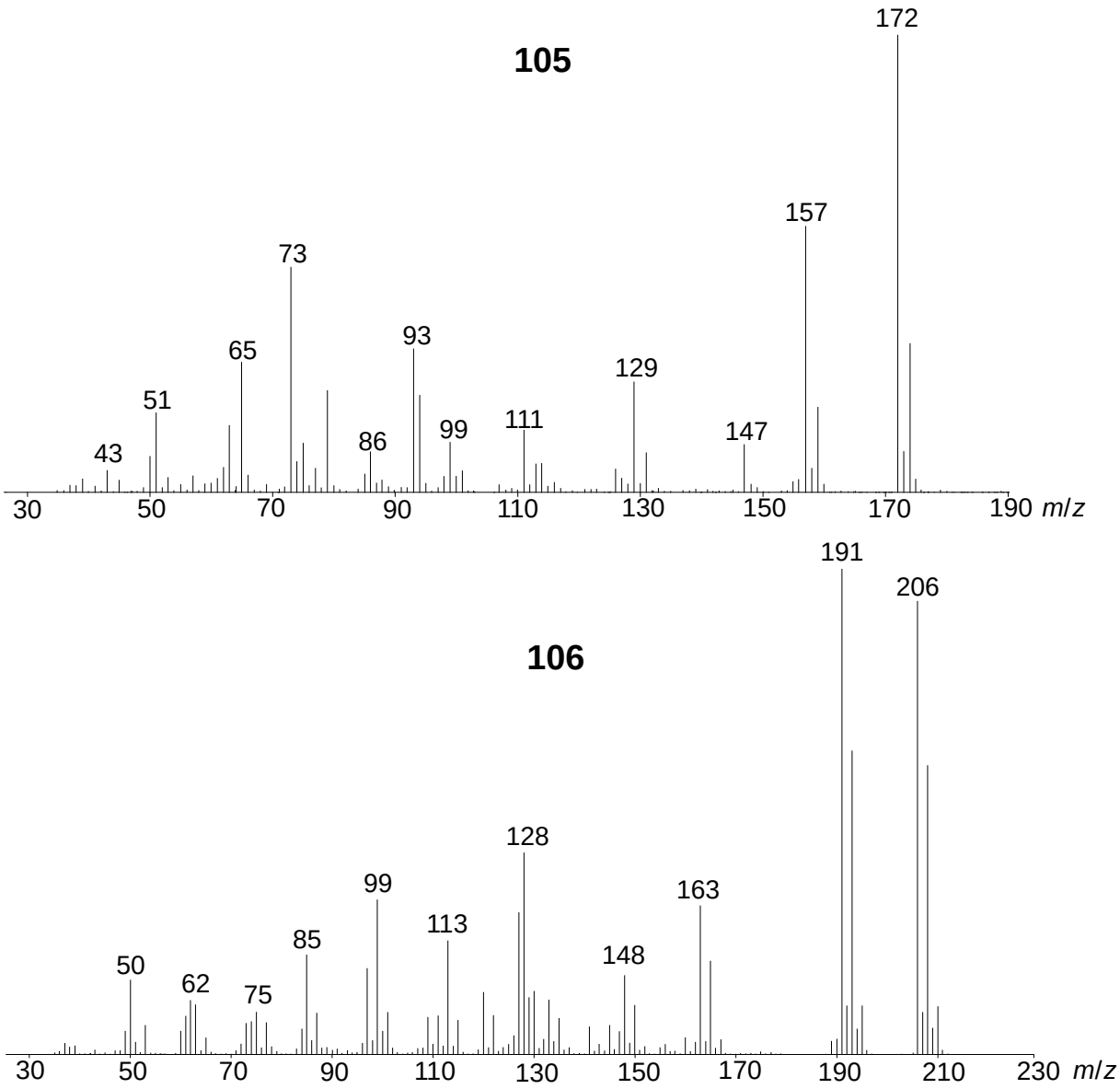


Figure 18 Mass spectra of volatile compounds **105** and **106**.[334]

The mass spectra of two unknown natural products (**105** and **106**) are shown in Figure 18. Due to their characteristic isotope patterns of the molecular ions, both compounds were assumed to contain one or two chlorine atoms. In comparison to mass spectra in electronic libraries, compound **105** was assigned as chlorodimethoxybenzenes while dichlorodimethoxybenzenes constituted substance **106**. Yet, this couldnot be the evidence for the unambiguous compound structure, since the differences of distinct constitutional isomers were comparably small. Therefore, it is necessary to concern other complementary criteria,

such as the comparison of GC retention indices of those two components to all possible structural isomers.

For this reason, six constitutional isomers of a chlorodimethoxybenzene (**105a**-**105f**) and eleven constitutional isomers (**106a**-**106k**) of a dichloromethoxybenzene were all obtained either via synthesis or purchased from commercial suppliers (Figure 19).

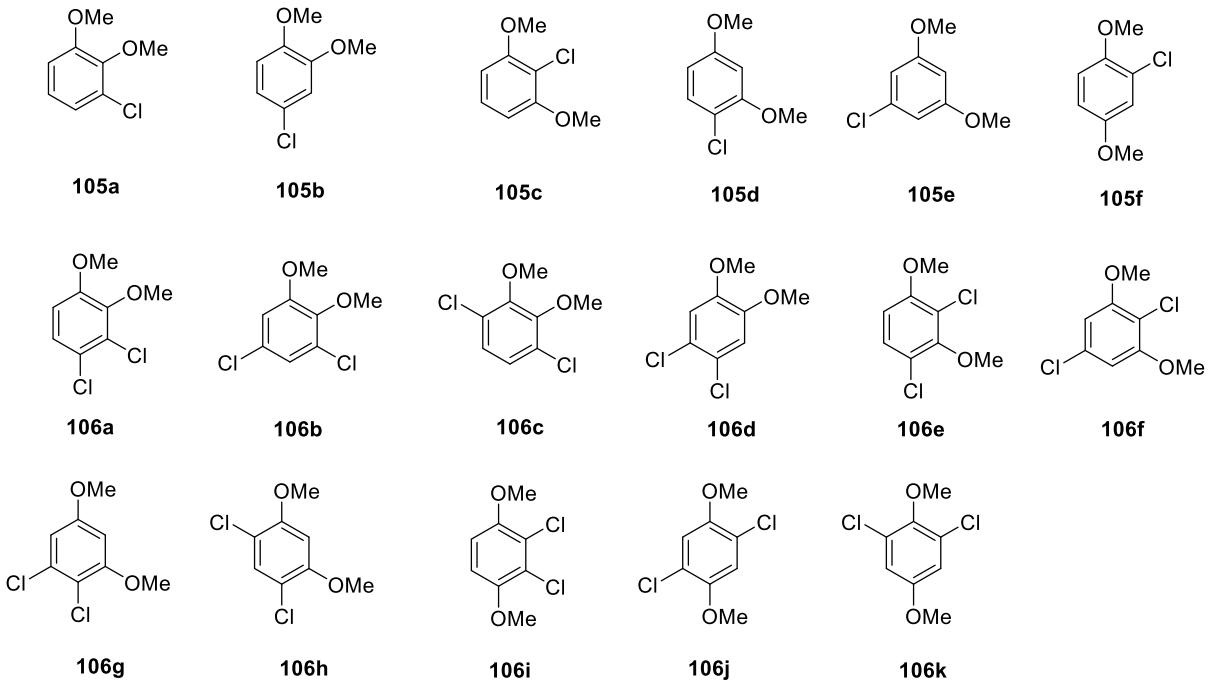
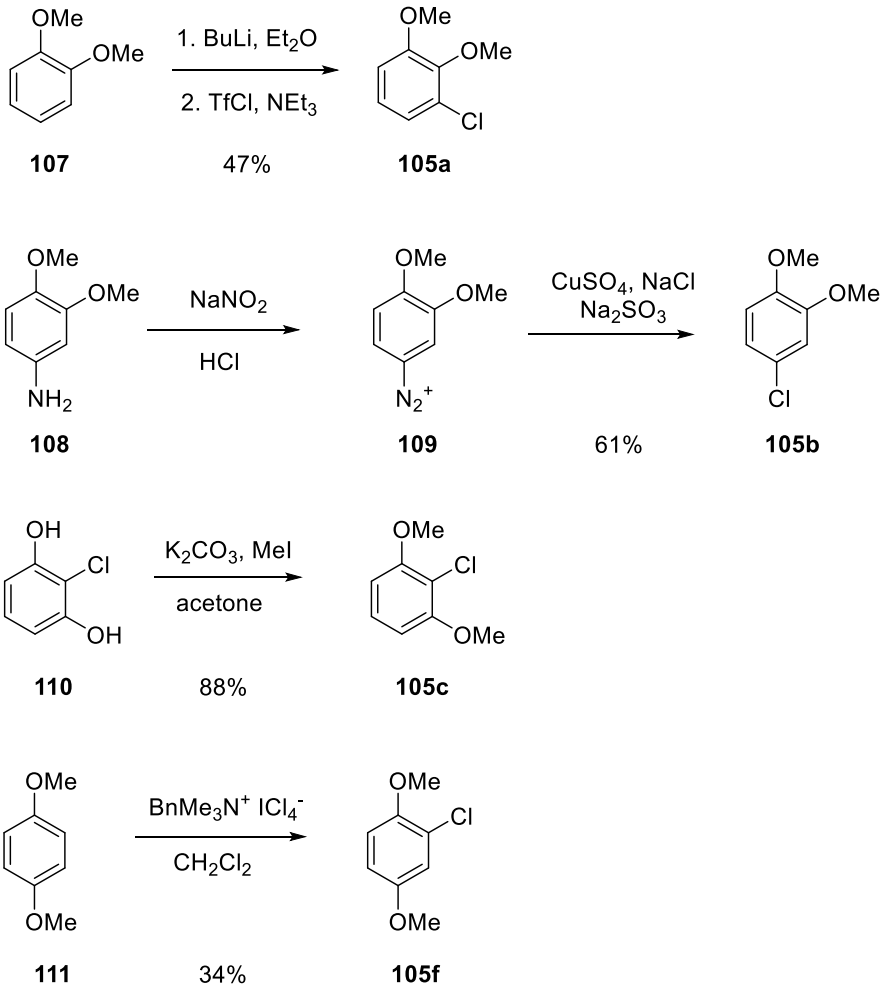


Figure 19 Constitutional isomers of chlorodimethoxybenzene **105** and dichlorodimethoxybenzene **106**.[334]

Among the candidates for determining the structure of chlorodimethoxybenzene, compounds **105d** and **105e** were commercially available, while the other regioisomers were afforded individually by synthesis representing in Scheme 8. *ortho*-Lithiation of veratrole (**107**) followed by chlorination with trifluoromethanesulfonyl chloride and triethylamine afforded compound **105a** with an acceptable yield (47%). 4-Aminoveratrole (**108**) was primarily converted into diazonium salt **109**, subsequently via a Sandmeyer reaction generated **105b** (60% over two steps). Double methylations of 2-chlororesorcinol

(**110**) with potassium carbonate and methyl iodide in acetone was able to produce isomer **105c** in a good yield (88%). The last isomer **105f** was achieved by chlorination of 1,4-dimethoxybenzene (**111**) with benzyltrimethylammonium tetrachloroiodate in moderate yield (34%).[335]

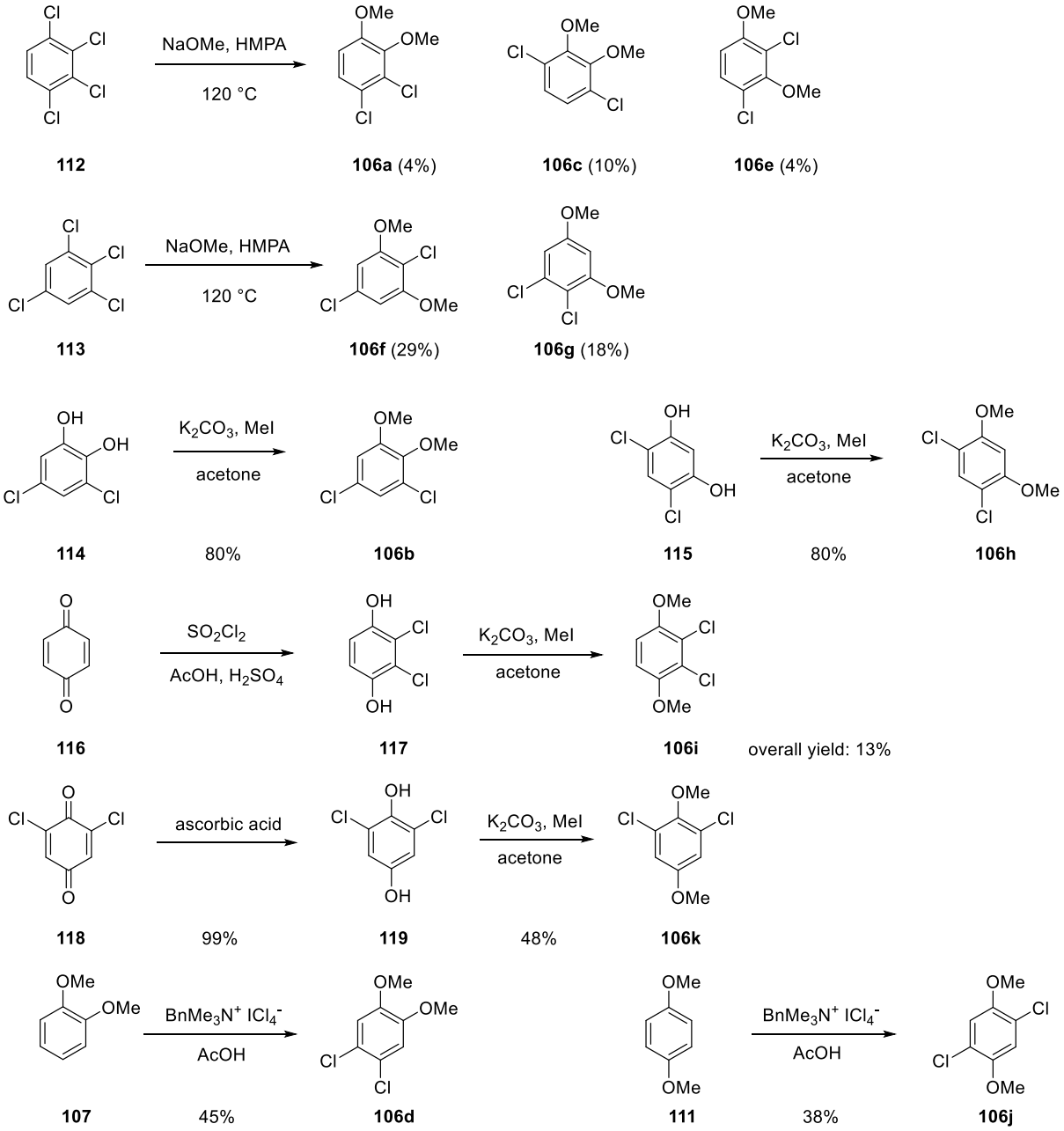


Scheme 8 Synthesis of chlorodimethoxybenzenes as reference compounds.[334]

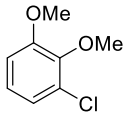
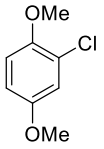
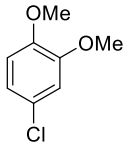
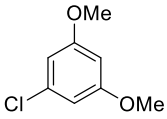
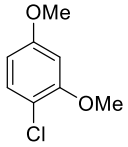
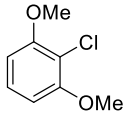
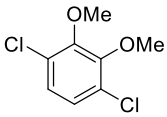
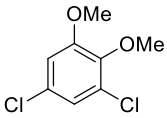
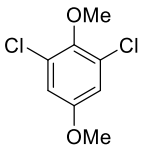
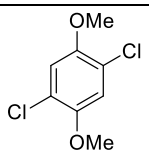
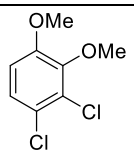
For dichlorodimethoxybenzene **106**, all possible constitutional isomers were synthesized and summarized in Scheme 9. Compounds **106a**, **106c**, and **106e** were side products arising from the reaction of 1,2,3,4-tetrachlorobenzene (**112**) with sodium methoxide in hot HMPA, which is described to manufacture 1,2,3-trichloro-4-methoxybenzene and 1,2,3-trichloro-3-methoxybenzene by monosubstitution.[336] Yet, disubstitution also took place and afforded a series of mixture, which were later

rigorously purified by repeated column chromatography. The same reaction condition could also bring the molecules **106f** and **106g** in better yields (29% and 17%). Methylation of the precursors, 3,5-dichloroactechol (**114**) and 4,6-dichlororesorcinol (**115**) by treatment with potassium carbonate and methyl iodide resulted in the isomers **106b** and **106h** in efficient yield (80%). Derivative **106i** was prepared in two steps, initially benzoquinone (**116**) was treated with sulfuryl chloride under acidic condition to yield 2,3-dichlorohydroquinone (**117**), then methylation to reach the target with 13% overall yield. Reduction of 2,6-dichorobenzoquinone (**118**) with ascorbic acid to the corresponding hydroquinone **119** further methylation developed **106k** in acceptable yield (48%). Isomers **106d** and **106j** can be obtained by chlorination of veratrole (**107**) and 1,4-dimethoybenzene (**111**) with two equivalents of benzyltrimethylammonium tetrachloroiodate respectively. Both reactions were done in moderate yields (45% and 38%).

All these constitutional isomers were examined by GC-MS analysis in application with the same GC column as for the headspace extract from *Geniculosporium* (HP5-MS). By comparison of mass spectra and retention indices (listed in Table 2), the structure of **105b** and **106b** were unambiguously assigned to the natural products **105** and **106** separately. Neither of them was discovered as natural products previously. On the contrary, compounds **105f** and **106k** have been investigated from the fungus *Bjerkandera adusta*.[337] In fact, the dichloromethoxybenzene (**106b** or **106k**) could be possibly derived from the monochloromethoxybenzene (**105b** or **105f**) via a second chlorination and hypothesized that the chlorinated compounds in both metabolites share one and the same biosynthetic pathway.[334]



Scheme 9 Synthesis of dichlorodimethoxybenzenes as reference compounds.[334]

Compound number	Structure	Compound name	*I* (HP5-MS)
105a		1-Chloro-2,3-dimethoxybenzene	1278
105f		2-Chloro-1,4-dimethoxybenzene	1292
105b		4-Chloro-1,2-dimethoxybenzene	1322
105e		1-Chloro-3,5-dimethoxybenzene	1340
105d		1-Chloro-2,4-dimethoxybenzene	1374
105c		2-Chloro-1,3-dimethoxybenzene	1404
Natural Product **105**			1322
106c		1,4-Dichloro-2,3-dimethoxybenzene	1339
106b		1,5-Dichloro-2,3-dimethoxybenzene	1426
106k		1,3-Dichloro-2,5-dimethoxybenzene	1443
106j		1,4-Dichloro-2,5-dimethoxybenzene	1448
106a		1,2-Dichloro-3,4-dimethoxybenzene	1466

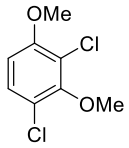
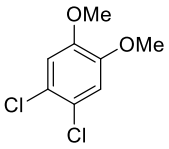
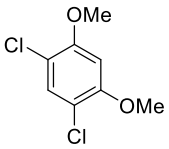
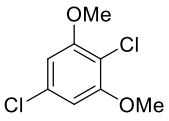
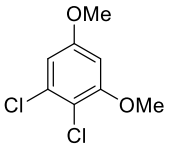
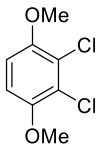
106e		1,3-Dichloro-2,4-dimethoxybenzene	1487
106d		1,2-Dichloro-4,5-dimethoxybenzene	1505
106h		1,5-Dichloro-2,4-dimethoxybenzene	1545
106f		2,5-Dichloro-1,3-dimethoxybenzene	1556
106g		1,2-Dichloro-3,5-dimethoxybenzene	1565
106i		2,3-Dichloro-1,4-dimethoxybenzene	1574
Natural Product **106**			1426

Table 2 GC retention indices of all constitutional isomers of chloromethoxybenzene, dichloromethoxybenzene and natural products **105** and **106**.[334]

In conclusion, 4-chloro-1,2-dimethoxybenzene (**105b**) and 1,5-dichloro-2,3-dimethoxybenzene (**106b**) were identified as volatile natural products (**105** and **106**) emitted by the fungus *Geniculosporium*. Their biological evaluation will be discussed in the following chapter.

2.2 Analysis of volatiles emitted by the fungal Xylariaceae

In this study, 14 fungal strains from the Xylariaceae family were analysed for their volatiles emitted by agar plate cultures grown on YMG medium (Yeast and Malt Extract with Glucose). The volatiles were also trapped on charcoal filters through the closed-loop stripping apparatus (CLSA) headspace technique.[333] After one day of collection, a solvent extract of the adsorbed filter was analyzed by GC-MS. The analytical results were acquired three times to prove its reproducibility. The compounds were identified based on their mass spectra and retention indices in comparison to literature data and commercially available or synthetic reference compounds. After all, a total of 88 volatile compounds from various classes were identified. A representative chromatogram of a headspace extract from *Daldinia clavata* STMA 06094 is shown in Figure 20. The chromatograoms for the other species are demonstrated in chapter 4.4.1, while the occurrence of volatile organic compounds is tabulated in chapter 4.4.2.

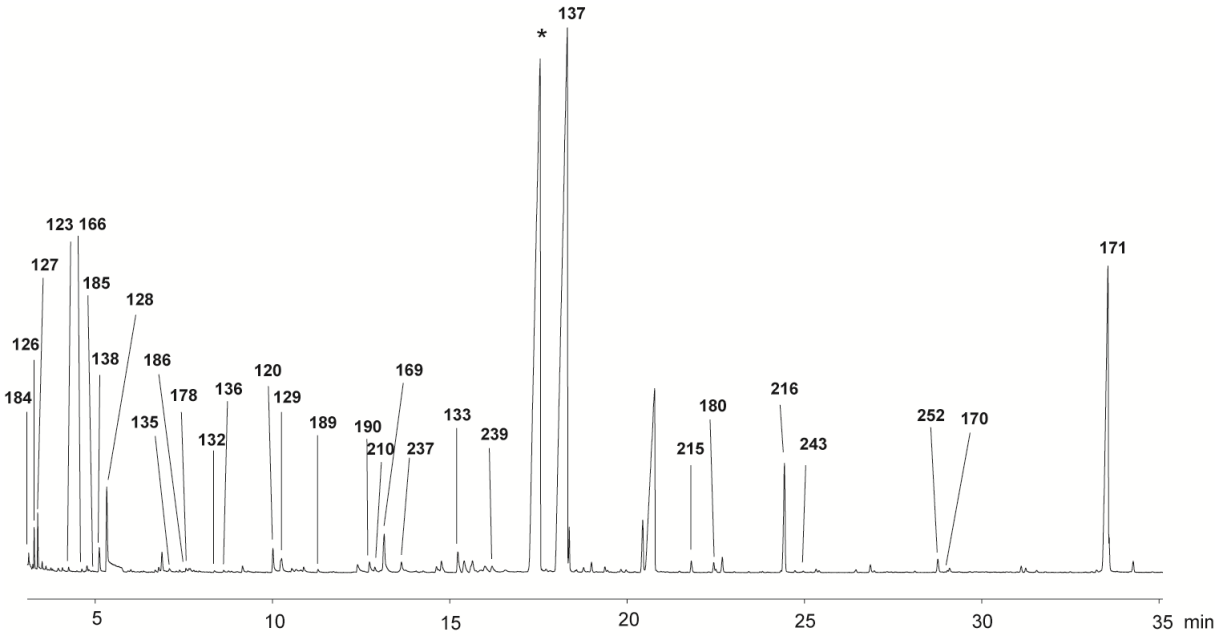
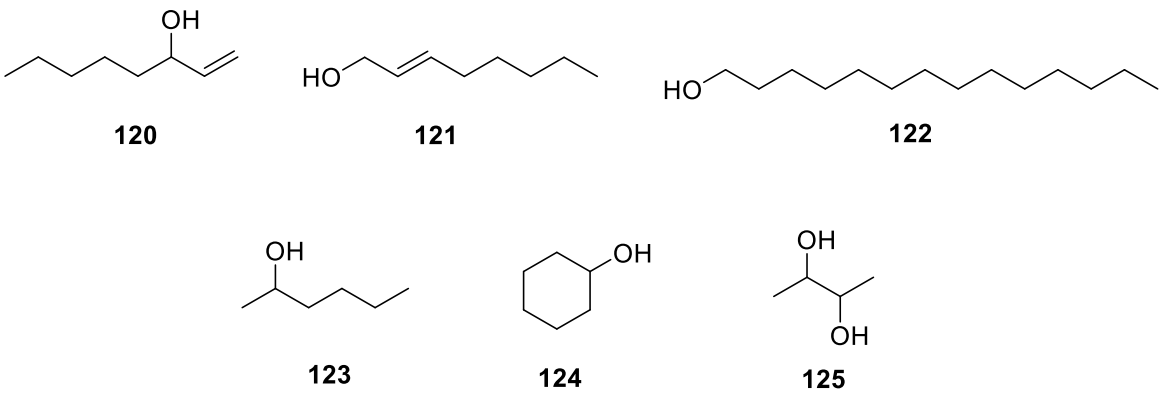


Figure 20 Total ion chromatogram of a representative headspace extract from *Daldinia clavata* STMA 06094. The peak labelled with an asterisk represents an unidentified natural product from *D. clavata*.

2.2.1 Fatty acid derivatives

2.2.1.1 Alcohols



120 **121** **122** **123** **124** **125**

Figure 21 Alcohols.

Among the fatty acid derivatives, several different alcohols were detected, including one primary alcohol (**122**) , one secondary alcohol (**123**), two unsaturated alcohols (**120** and **121**), one cycloalkanol (**124**) and one diol (**125**) (Figure 21). 1-Octen-3-ol (**120**) was present in the

following fungi, *Daldinia clavata*, *D. childiae*, *D. australis*, *D. eschscholzii*, *D. novae-zelandiae* and *D. concentrica*. But only two strains *D. australis* and *D. eschscholzii* emitted 2-octen-1-ol (**121**). Both volatiles are produced as microbial volatile organic compounds (MVOC's) during the primary fungal metabolism.[338] In particular, 1-octen-3-ol (**120**) is considered as the major product arising from enzymatic break-down of linoleic acid[339] and possesses the flavour of the mushrooms *Agaricus bisporus* and *Pleurotus* species.[275],[340] The other metabolites can be also found in these organisms. 1-Tetradecanol (**122**) occurred in *D. australis*. 2-Hexanol (**123**) was detected in *D. clavata*, well known as typical fungal smell.[277] Cyclohexanol (**124**) was only generated in strain *D. childiae*. This cycloalkanol plays an important role in inhibition of *Sclerotinia sclerotiorum* spore germination and mycelium growth.[341] In addition, one diol, 2,3-butanediol (**125**) was emitted by *D. hawksworthii*. This volatile compound is able to promote the growth and induce systemic resistance in *Arabidopsis thaliana.*[342],[343] It is also revealed that this diol can induce the plant production of nitric oxide (NO) and hydrogen peroxide either under normal circumstance or under drought stress.[344],[345]

2.2.1.2 Aldehydes and ketones

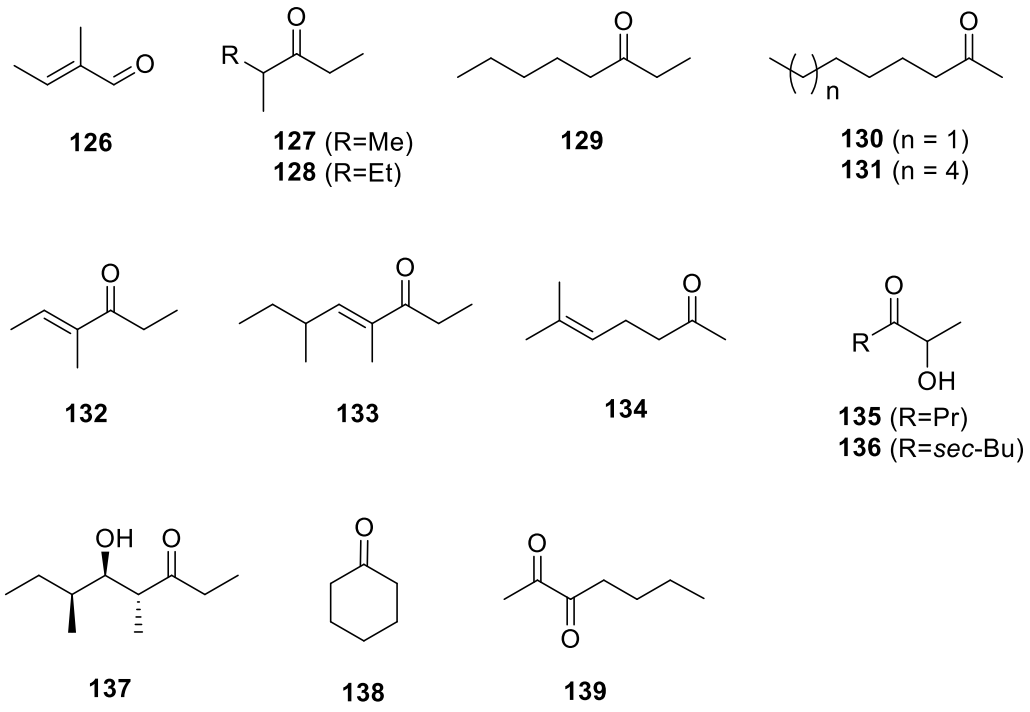


Figure 22 Aldehyde and ketones.

One aldehyde (**126**) and several ketones (**127**-**131**) were identified, in addition to unsaturated ketones (**132**-**134**), hydroxyketones (**135**-**137**), cyclic ketone (**138**) and diketone (**139**) (Figure 22). Tiglic aldehyde (**126**) occurred only in the fungal strain *D. clavata*. The methyl branched ketones, 2-methyl-3-pentanone (**127**) and 4-methyl-3-hexanone (**128**) were widespread in the *Daldinia* species, along with other typical fungal metabolites,[346] 3-octanone (**129**), 2-octanone (**130**) and 2-undecanone (**131**). Among the unsaturated ketones, (*E*)-4-methylhex-4-en-3-one (**132**) was identified in the strains *D. clavata*, *D. novae-zelandiae* and *D. concentrica*. Ketone **133** was dominated in *D. clavata* and *D. childiae*. It can be also named as manicone, which exists as an ant alarm pheromone from two north American species of ants *Manica mutica* and *M. bradleyi* (Hymenoptera, Formicidae).[347] *D. novae-zelandiae* released 6-methyl-5-hepten-2-one (**134**) derived from fatty acid biosynthesis.[348] Besides, 2-hydroxyhexan-3-one (**135**) and 2-hydroxy-4-methylhexan-3-

one (**136**) accompanied with acetyl valeryl (**139**), appeared frequently in *D. clavata*, *D. novae-zelandiae* and *D. concentrica*. They were investigated previously as volatile compounds from Gram-positive bacteria, *Corynebacterium glutamicum*.[349] (4*R*,5*R*,6*S*)-5-Hydroxy-4,6-dimethyloctan-3-one (**137**) was first time discovered as natural product in fungi family and participates in total synthesis of (-)-ebelactone A and B[350] and Auripyrone A.[351] It was distributed in almost every investigated Xylariaceae species. In addition, cyclohexanone (**138**) was found in trace amount in *D. childiae* and *Biscogniauxia cylinderispora* respectively.

2.2.1.3 Identification of volatiles 133 and 137 via synthesis

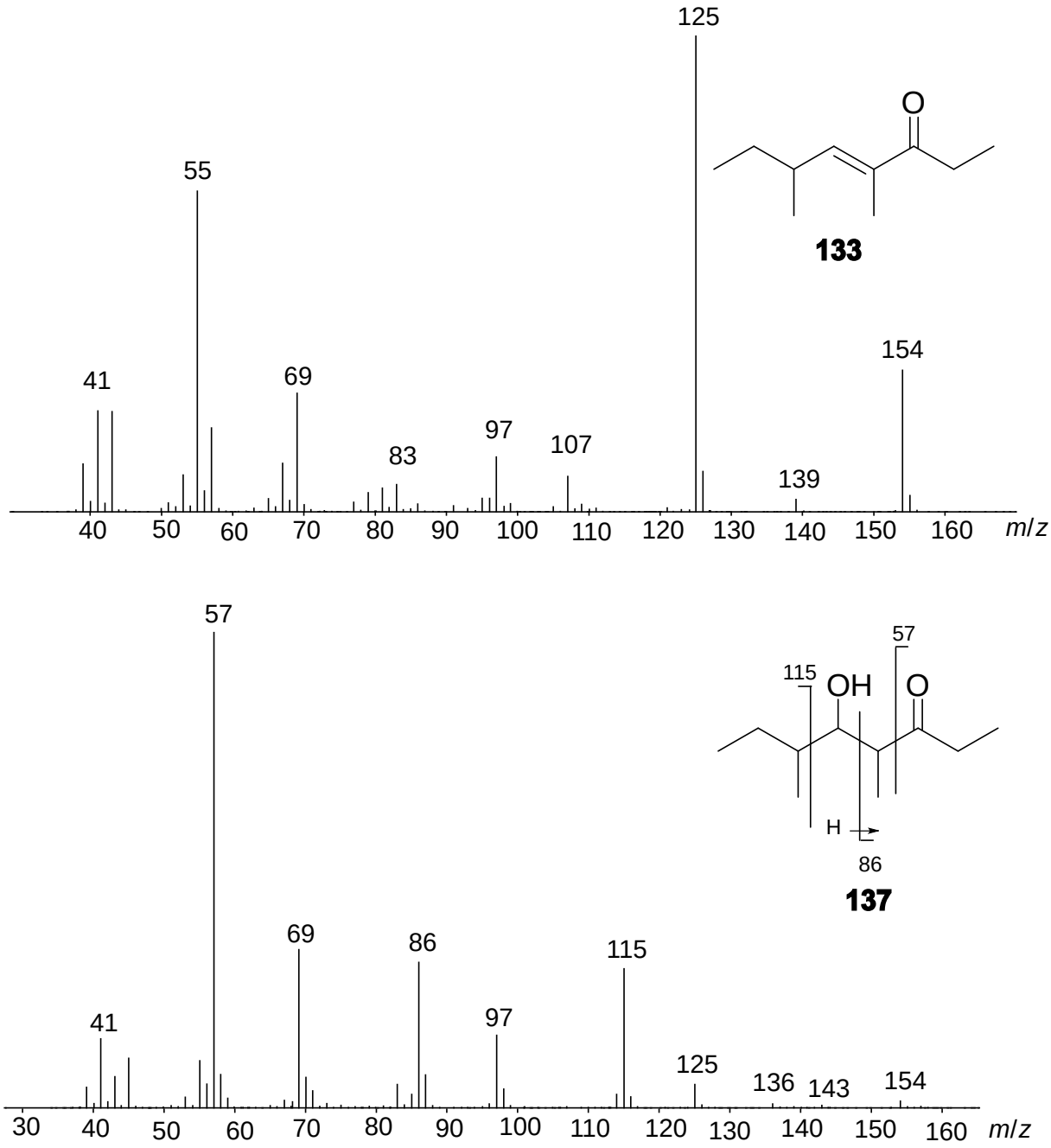
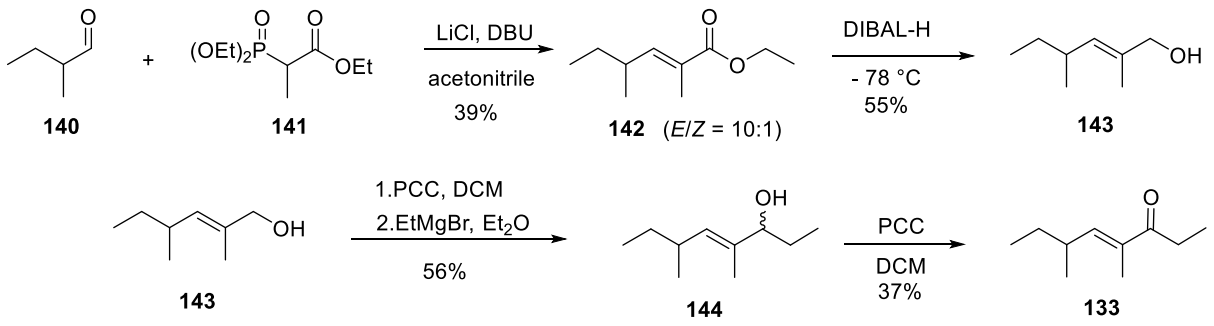


Figure 23 Mass spectra of natural products **133** and **137**.[352]

The absolute structural configuration of volatile organic compounds cannot only rely on the proposals based on GC-MS analysis data. In some cases, it is necessary to pursue reference compounds via chemical synthesis. The mass spectrum of compound **133** revealed characteristic fragment ions at *m*/*z* = 154 (molecular ion M^+), *m*/*z* = 139

(loss of methyl group), *m*/*z* =125 as base peak indicating an ethyl branching and *m*/*z* = 97 (cleavage of *sec*-butyl group). The chemical formula can be concluded as $C_{10}H_8O$ and shares a similar data base spectrum of manicone, (*E*)-4,6-dimethyloct-4-en-3-one. However, no available retention index for this substance was found in literature. Therefore, a total synthesis of compound **133** was planned from 2-methylbutanal (**140**) and triethyl 2-phosphonopropionate (**141**) by the treatment with lithium chloride and DBU in acetonitrile through a Horner-Wadsworth-Emmons reaction,[353] having ethyl (*E*)-2,4-dimethylhex-2-enoate (**142**) as a separable mixture of *E* and *Z* stereoisomers (*E*/*Z* = 10:1), subsequently reduced by diisobutylaluminium hydride into corresponding alcohol (**143**) with an acceptable yield (55%),[354] oxidised by pyridinium chlorochromate[355] and introduced the alkyl side chain via Grignard reaction yielding 56% of compound **144**, ultimately treated with another pyridinium chlorochromate oxidation in moderate yield (37%) (Scheme 10). The overall yield of 5 steps synthesis to reach the target molecule is only 1%. One explanation is that all the intermediates involved have sincerely low molecular weight leading to strong volatility in air, thereby the loss of products obviously existed during the operations.



Scheme 10 Synthesis of compound **133**.[352]

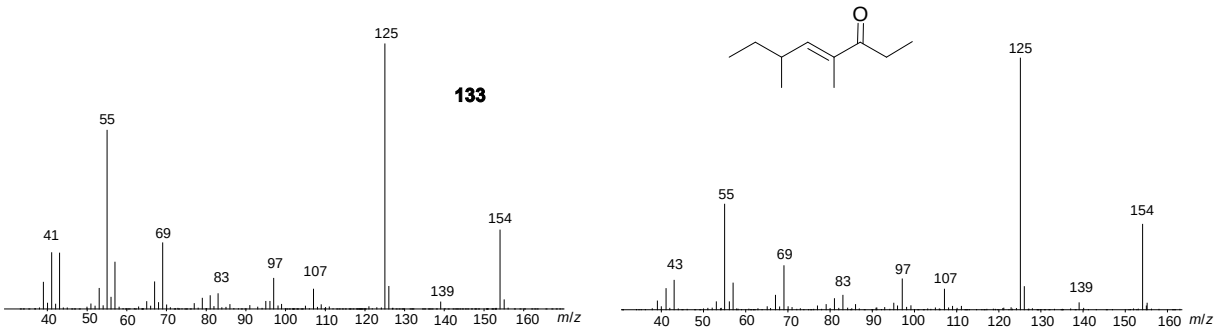
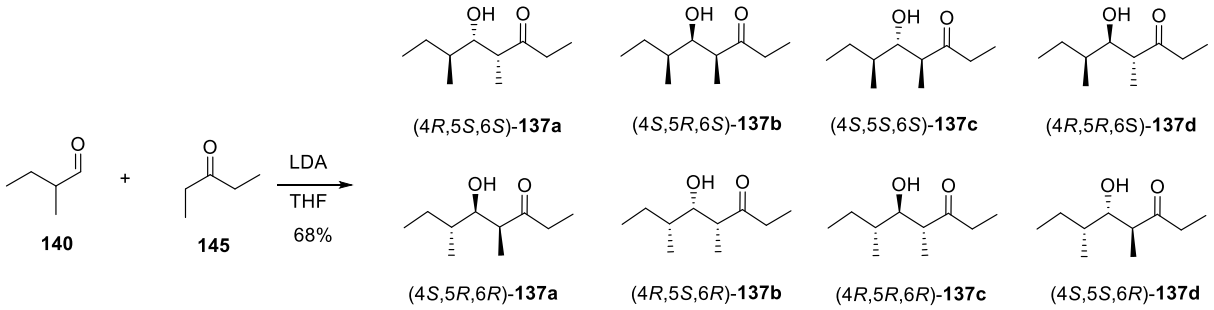


Figure 24 Mass spectra of natural product **133** and its authentic synthetic sample.

The mass spectra between natural product and authentic sample are shown in Figure 24 and share small differences, for instance, fragment ions at *m*/*z* = 41 and *m*/*z* = 55 indicate higher intensity in natural product than synthetic sample. This could be the background interference from the machine. Their retention indices are very close to each other, 1136 from natural product and 1140 from synthetic substrate, respectively. Based on these observations, compound **133** was assigned as manicone.

On the other hand, the mass spectrum of ketone **137** indicated some fragment ions which were also detected in **133** (Figure 23). This might suggest that both metabolites are structurally related. The fragments ions at *m/z* = 57 as a base peak and *m/z* = 86 result from α-cleavage and McLafferty rearrangement of 4-methyl-3-ketone. The fragment ion at *m/z* = 115 that may result from an α-cleavage next to the alcohol function supported the molecular structure as a hydroxy-ketone. The fragment ion at *m*/*z* = 154 results from the dehydration of hydroxyl group and the fragment ion at *m*/*z* = 143 arises from the cleavage of alkyl group from the molecular ion. The higher retention time of compound **137** in comparison of **133** offers the hint that via a dehydrolation of hydroxyl-ketone **137**, the C=C double bond in **133** can be installed as expected.[352] Therefore, the molecular formula for hydroxyl-ketone **137**

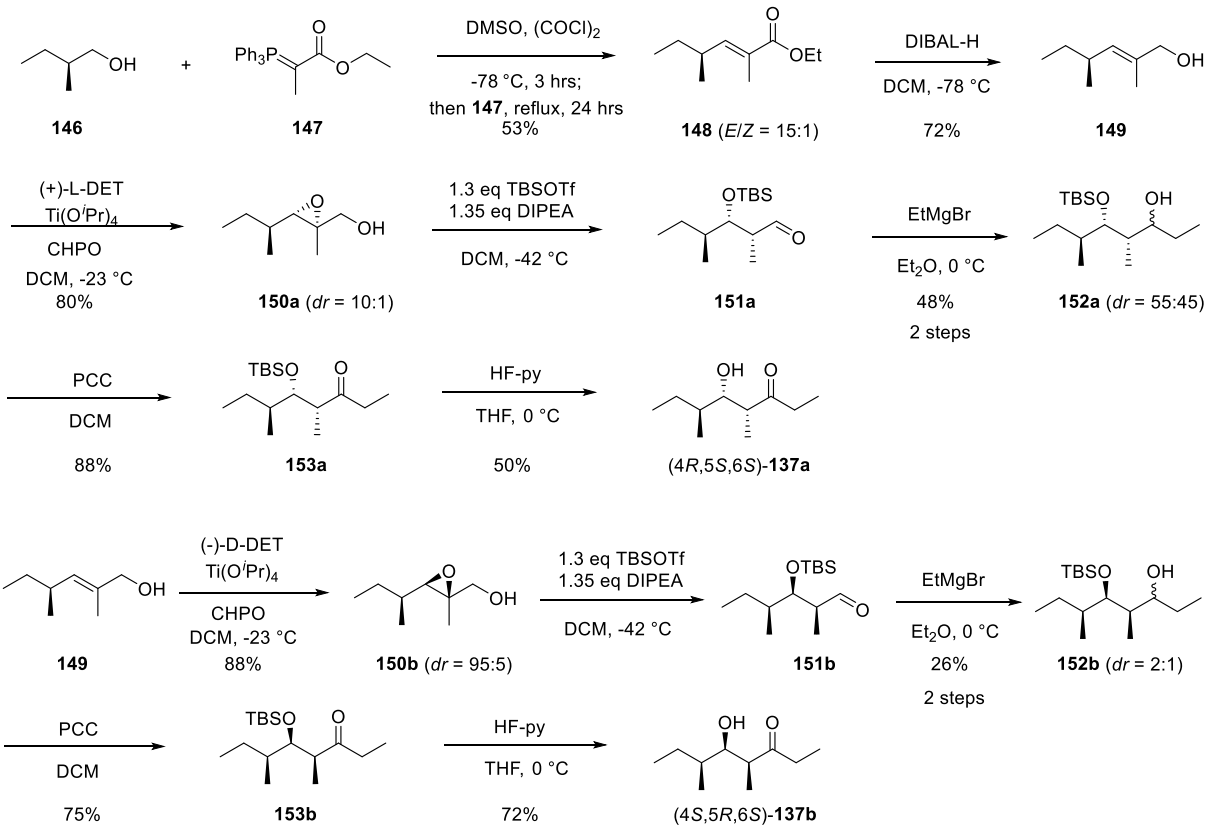
can be assigned as $C_{10}H_{20}O_2$. To verify the structural proposal, an aldol addition between racemic 2-methylbutanal (**140**) and pentan-3-one (**145**) was carried out and generated a racemic mixture of all four diastereomers **137a**-**d** (Scheme 11).[356] All eight stereoisomers were successfully separated by GC on a homochiral stationary phase performed with a CP-ChiraSil-Dex CB capillary column, one of which was identified as natural product **137** in comparison of retention time and mass spectra (Figure 25).



Scheme 11 Synthesis of a racemic mixture of all four diasteromers of **137**.[352]

In order to understand the relative and absolute configuration of the natural product **137**, an enantioselective synthesis was initially conducted (Scheme 12). It began with (*S*)-(-)-2-methylbutanol (**146**) via Swern oxidation with oxalyl chloride and DMSO at low temperature and was converted into the corresponding aldehyde, followed by one-pot Wittig reaction with ethyl 2-(triphenylphosphoranylidene)propionate (**147**) under reflux yielded the esters (2*E*,4*S*)- and (2*Z*,4*S*)-**148** as a mixture of diastereomers (approximately 15:1) which were separated by repeated column chromatography.[357] Reduction of *E*-ester with diisobutylaluminium hydride afforded the corresponding alcohol (**149**) with an acceptable yield (72%).[354] Sharpless epoxidation introduced the epoxides (**150a** and **150b**) enantioselectively by (+)-diethyl L-tartrate and (-)-diethyl D-tartrate, (80% and 88%, respectively).[358] Afterwards, treated with *tert*-butyldimethylsilyl trifluoromethanesulfonate and Hünig's base,

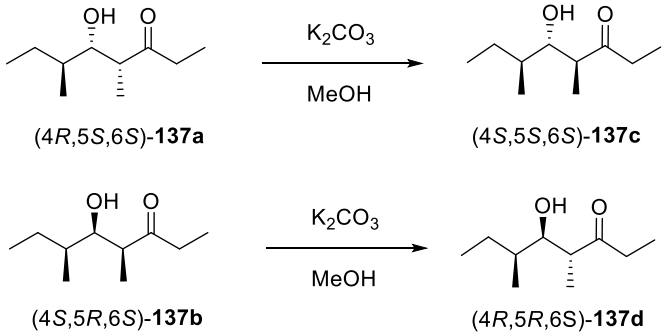
epoxide rings were opened, followed by hydride migration to reach aldehydes (**151a** and **151b**).[359] These aldehydes were extremely unstable in air and can be further oxidized into carboxylic acids. Therefore, direct addition of ethyl group at C-2 position with Grignard reagent achieved compounds **152a** and **152b** with moderate yields over two steps (48% and 26%, separately). Subsequently, oxidation by pyridinium chlorochromate gave the access to **153a** and **153b** in high yields (88% and 75%). At last, deprotection with hydrogen fluoride in pyridine at 0 °C formed 50% of (4*R*,5*S*,6*S*)-**137a** and 72% of (4*S*,5*R*,6*S*)-**137b**.[360] The overall yields of 7 synthetic steps to reach both isomers (4*R*,5*S*,6*S*)-**137a** and (4*S*,5*R*,6*S*)-**137b** were 6% and 7% respectively.



Scheme 12 Enantioselective synthesis of (4*R*,5*S*,6*S*)-**137a** and (4*S*,5*R*,6*S*)-**137b**.[352]

The same approach starting from (2*Z*,4*S*)-**148** could also give access to two more stereoisomers, (4*S*,5*S*,6*S*)-**137c** and (4*R*,5*R*,6*S*)-**137d**.

However, the *Z*-ester couldnot be reached in sufficient quantity for completing the further synthetic sequence. Therefore, (4*S*,5*S*,6*S*)-**137c** and (4*R*,5*R*,6*S*)-**137d** were afforded by epimerisations at the α-carbon (C-4) of (4*R*,5*S*,6*S*)-**137a** and (4*S*,5*R*,6*S*)-**137b** under mildly basic condition, respectively (Scheme 13).



Scheme 13 Epimerisations of (4*R*,5*S*,6*S*)-**137a** and (4*S*,5*R*,6*S*)-**137b** under basic conditions.[352]

Four newly synthesized compounds ((4*R*,5*S*,6*S*)-**137a**, (4*S*,5*R*,6*S*)-**137b**, (4*S*,5*S*,6*S*)-**137c** and (4*R*,5*R*,6*S*)-**137d**) were added to the synthetic mixture of all eight stereoisomers separately and the analysis of enantioselective GC demonstrated that the natural product was assigned to (4*R*,5*R*,6*S*)-**137d** in terms of retention time and mass spectra (Figure 25).

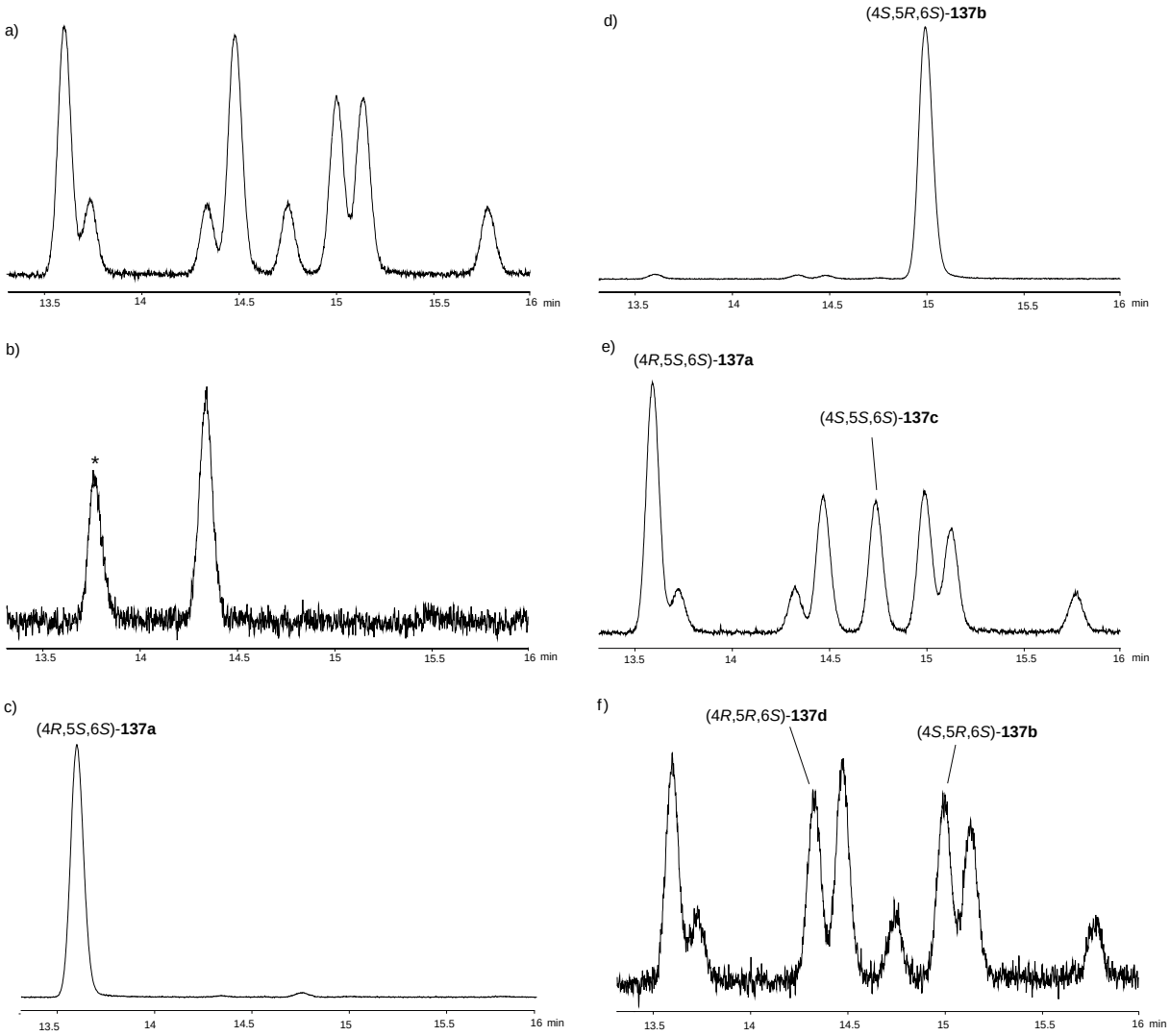


Figure 25 Gas chromatographic analysis of **137** on a homochiral stationary phase performed with a CP-ChiraSil-Dex CB capillary column. a) the synthetic mixture of all eight stereoisomers (Scheme 11), b) natural product form D. clavata, c) enantioselectively synthesized (4*R*,5*S*,6*S*)-**137a** (Scheme 12), d) enantioselectively synthesized (4*S*,5*R*,6*S*)-**137b** (Scheme 12), e) coinjection of a) and epimersation product (4*S*,5*S*,6*S*)-**137c** (Scheme 13) and f) coinjection of a) and epimersation product (4*R*,5*R*,6*S*)-**137d** (Scheme 13). The asterisk labelled peak represents the second main volatile organic compound in the headspace extracts from *D. clavata*, also showed in Figure 20.[352]

Additionally, the chemical characterisation of all eight stereoisomers of **137** obtained by aldol addition (Scheme 11) could be accomplished by extensive chromatographic purification. First and foremost, via column chromatography on silica gel, one pair enantiomer **137a** were excluded from the racemic mixture. Preparative reversed phase HPLC succeeded

in separating other three compounds **137b**, **137c** and **137d**. Furthermore, preparative HPLC on a homochiral stationary phase refined the pure enantiomers of these compounds, expect **137a**. Fortunately, enantiomerocally pure (4*R*,5*S*,6*S*)-**137a** was produced by enantioselective synthesis as described above, and the peaks of the enantiomers of **137a** in the GC chromatogram on a homochiral stationary phase could be distributed individually by comparsion to synthetic authenic sample (4*R*,5*S*,6*S*)-**137a**. At last, the structures of all eight peaks in chiral GC analysis could be determined and proved again the structure of (4*R*,5*R*,6*S*)-**137d** as the volatile organic compound released from *D. clavata* (Figure 26). A table of the ^{13}C NMR data of all four stereoisomers **137a**-**d** are summarised below.

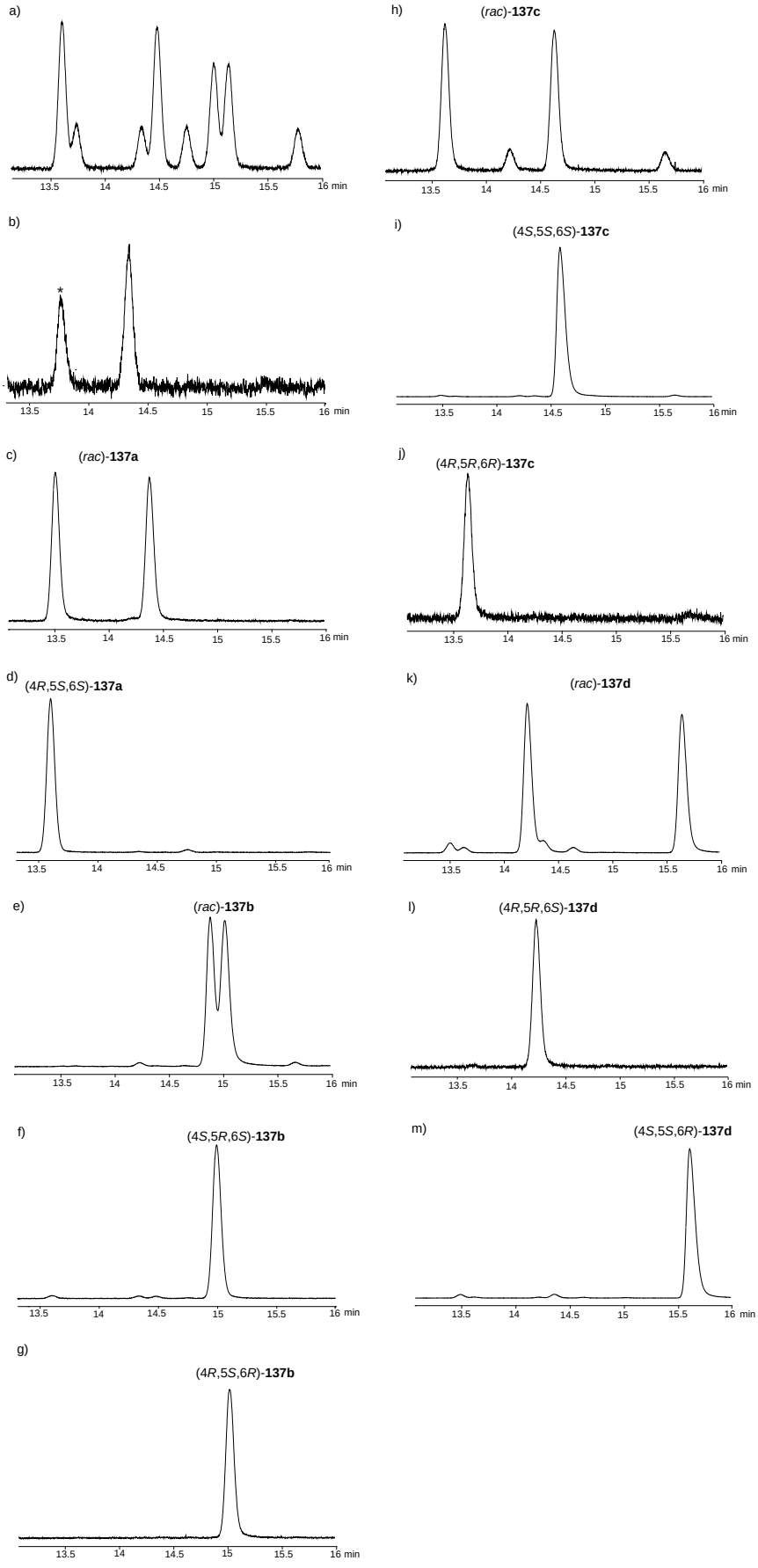
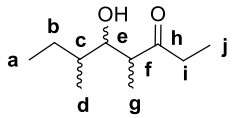

a)
13.5 14 14.5 15 15.5 16 min
h)
(*rac*)-**137c**
13.5 14 14.5 15 15.5 16 min
b)
*
13.5 14 14.5 15 15.5 16 min
i)
(4*S*,5*S*,6*S*)-**137c**
13.5 14 14.5 15 15.5 16 min
c)
(*rac*)-**137a**
13.5 14 14.5 15 15.5 16 min
j)
(4*R*,5*R*,6*R*)-**137c**
13.5 14 14.5 15 15.5 16 min
d)
(4*R*,5*S*,6*S*)-**137a**
13.5 14 14.5 15 15.5 16 min
k)
(*rac*)-**137d**
13.5 14 14.5 15 15.5 16 min
e)
(*rac*)-**137b**
13.5 14 14.5 15 15.5 16 min
l)
(4*R*,5*R*,6*S*)-**137d**
13.5 14 14.5 15 15.5 16 min
f)
(4*S*,5*R*,6*S*)-**137b**
13.5 14 14.5 15 15.5 16 min
m)
(4*S*,5*S*,6*R*)-**137d**
13.5 14 14.5 15 15.5 16 min
g)
(4*R*,5*S*,6*R*)-**137b**
13.5 14 14.5 15 15.5 16 min

Figure 26 Gas chromatographic analysis of **137** on a homochiral stationary phase performed with a CP-ChiraSil-Dex CB capillary column. a) the synthetic mixture of all eight stereoisomers (Scheme 11), b) natural product from *D. clavata*, and c)- m) pure racemates and enantiomers of **137a**-**d**. Compound (4*R*,5*S*,6*S*)-**137a** was afforded by enantionselective synthesis (Scheme 12), the pure racemates of **137a**-**d** and the pure enantiomers of **137a**, **137b** and **137d** were obtained by chromatographic separation from the synthetic mixture of all eight stereoisomers. The asterisk labelled peak stands for the second main volatile organic compound in the headspace extracts from *D. clavata*, also showed in Figure 20.[352]

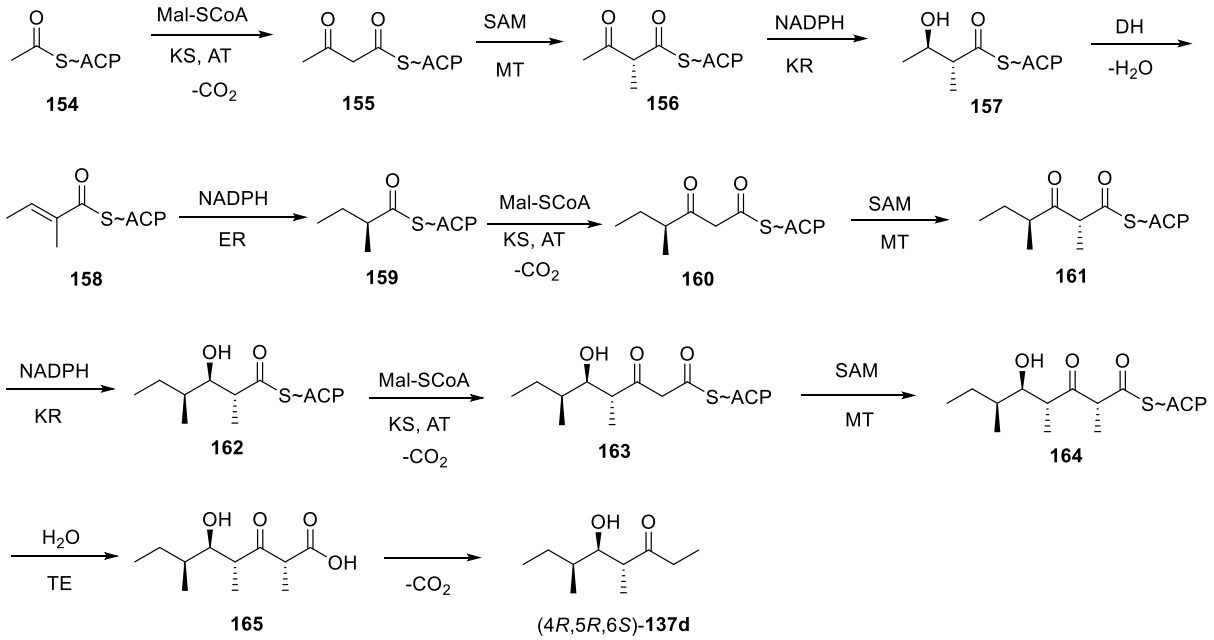


^{13}C NMR (ppm)	137a	137b	137c	137d
a	9.3	11.2	11.8	12.0
b	25.4	26.2	23.6	27.1
c	37.3	37.5	38.1	37.0
d	15.0	14.5	16.3	13.9
e	74.6	74.7	78.5	75.8
f	47.3	48.2	47.7	48.8
g	11.3	11.4	14.8	12.5
h	215.4	214.5	215.7	215.0
i	34.6	34.9	36.2	36.1
j	7.8	7.8	7.7	7.8

Table 3 Carbon NMR spectroscopic data (chemical shifts in ppm) for **137a**-**d** (125 MHz, C_6D_6).

The biosynthetic pathway to (4*R*,5*R*,6*S*)-**137d** can be assumed as a typical fungal iterative polyketide synthase (PKS). As represented in Scheme 14, acryl-carrier-protein (ACP) bounding to acetate as a starter, first elongation occurs with malonyl-SCoA (Mal-SCoA) under the catalysis of of an acyl transferase (AT) and a ketosynthase (KS) domain and affords acetoacetyl-SACP. Later, SAM-dependent C-methylation by a methyl transferase domain (MT) might be performed to introduce the stereochemical course in the 4*R*-configuration for **156**. The same

stereochemical course should continuously proceed in the coming chain extension steps as an iterative PKS takes place. A hydroxyl group is added at C-3 position by a keto-reductase (KR), followed by elimination of water by a dehydratase domain (DH) and reduction of the C=C double bond by an enoyl reductase (ER) to install the stereocenter in **159**. Subsequently, another chain extension with malonyl-SCoA and methylation introduces a second stereocenter in **161**. The final stereochemical course for target molecule is completed by reduction of **161** with KR into corresponding alcohol **162**. The last extension is also carried out by malonyl-SCoA and methylation to reach intermediate **164**. Afterwards, a thioesterase (TE) converts **164** into the β-keto acid **165**, which is spontaneously decarboxylated into (4*R*,5*R*,6*S*)-**137d**.[352] Further investigations are necessarily demanded to confirm this hypothetical PKS biosynthesis.



Scheme 14 Proposed biosynthetic pathway to (4*R*,5*R*,6*S*)-**137d**.[352]

2.2.1.4 Esters

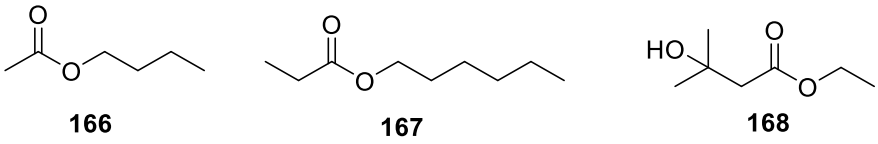


Figure 27 Esters (**166**-**168**).

The production of volatile fatty acids and fatty acid esters in the fermentation of the yeast *Saccharomyces cerevisiae* has been studied and several hypotheses about the mechanism of the ester synthesis have been published.[361] Two main flavour active esters, the acetate esters and the medium-chain fatty acid (MCFA) ethyl esters, remain in the fermented beverages.[361] In our case, butyl acetate (**166**) as a typical apple aroma and flavour volatile,[362] was found in *D. clavata* in trace amount. It can reduce the conidial adhesion and germination from fungus *Botrytis cinerea* which leads to apple fruit rot.[362] Another flavour volatile fraction hexyl propionate (**167**) can be observed in *D. australis*. The β-hydroxy ester **168** likely arising from leucine carbon skeleton was detectable in strain *Biscogniauxia cylinderispora*.

2.2.2 Lactones

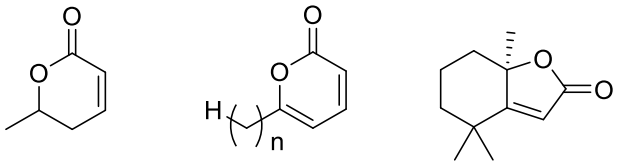


Figure 28 Lactones (**169**-**172**).

Fungi can produce a variety of lactones as the 3-hydroxylation of fatty acids and even 4- or 5-hydroxylations.[363] *Trichoderma viride* produces the well-known coconut odor lactone, 6-pentyl-α-pyrone.[364] In our earlier

research to the soil fungi *Trichoderma viride* and *Trichoderma asperellum*, several new alkyl- and alkenyl-*2H*-pyran-2-ones were examined and unambiguously synthetically varified.[365] Specially, two lactones are widely distributed in the fungal volatile organic compounds. 6-Methyl-5,6-dihydro-2*H*-pyran-2-one (**169**) owns the predominate peak in *D. eschscholzii* and *D. concentrica*, while it occurs in small amounts in *D. clavata*, *D. cf. caldarium* and *D. novae-zelandiae*. It was firstly discovered in 1859 from steam distillation of European mountain ash berries, *Sorbus aucuparia* (*Rosaceae*).[366] 6-Heptyl-2*H*-pyran-2-one (**170**) could be only observed in *D. clavata* and *D. childiae*, while its longer alkyl chain derivative, 6-pentyl-α-pyrone derivative (**171**) is extremely abundant in *D. clavata*, *D. childiae* and *D. australis* and detectable in *D. novae-zelandiae* and *Hypoxylon* sp. nov. JF 11167.

This fungal family is also producing other lactones as minor products. Benzofuran (**172**) was generated only in the fungal strain *H. macrocaipum*. It also constitutes one of the two major components from the essential oil of *Viola odorata* Linn (Violaceae) leaves.[367] This medicinal plant is able to cure bronchitis, digestive disorders, postoperative tumor metastasis, diabetes and cancer.[367]

2.2.2.1 Structural elucidation of two lactones 169 and 171

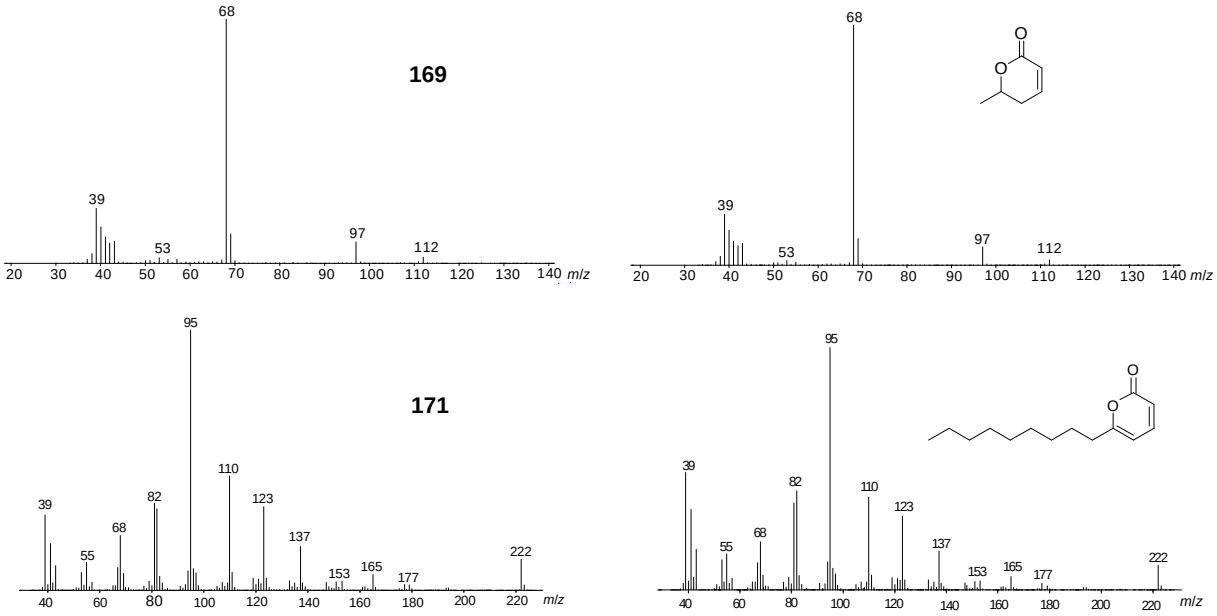
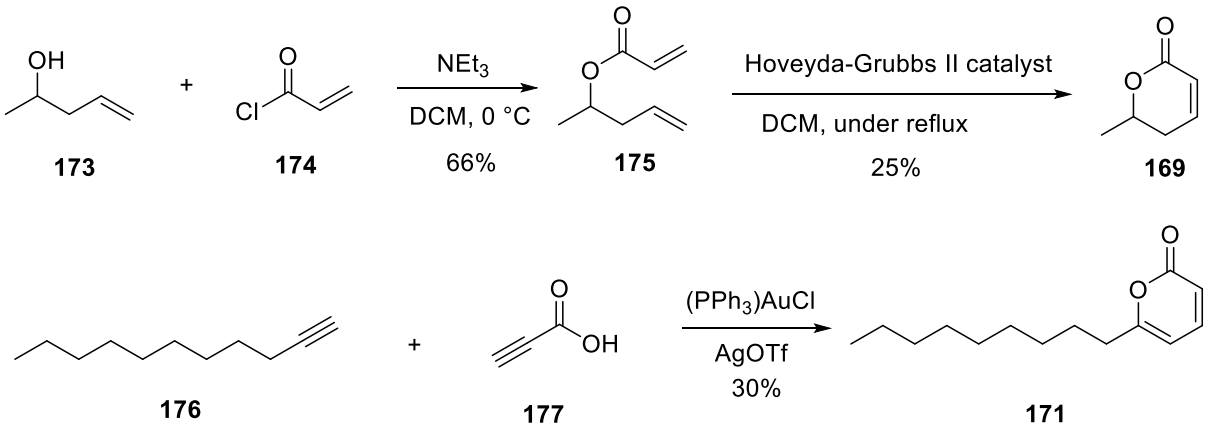


Figure 29 Mass spectra of two Lactones **169** and **171** and the authentic synthetic samples.

The mass spectra of the two lactones **169** and **171** give some clue in their structural elucidation (Figure 29). The molecular ion of compound **169** occurs at *m*/*z* = 112. The loss of the methyl branch shows fragment ion at *m*/*z* = 97. The base peak at *m*/*z* = 68 can be explained either by cleavage of carbon dioxide or by loss of acetaldehyde. From the former ion, the methyl group is cleavaged and results in the fragment ion at *m*/*z* = 53. The singal at *m*/*z* = 39 arises from the latter ion through the cleavage of carbon monoxide. This suggested the structure of 6-methyl-5,6-dihydro-2*H*-pyran-2-one as natural product **169**.

The similar mass fragment mechanism can be applied in the explanation of compound **171**. Fragment ion at *m*/*z* = 95 appears as the base peak. The peaks at *m*/*z* = 110, 123, 137 and 165 are all derived from the cleavage of the alkyl branch chain at different points. The singal at *m*/*z* = 222 stands for the molecular ion, from which the loss of carbon dioxide explains the fragment ion at *m*/*z* = 177, followed by elimination of C_2H to obtain the ion at *m*/*z* = 153. On the other hand, after cleavage of the alkyl

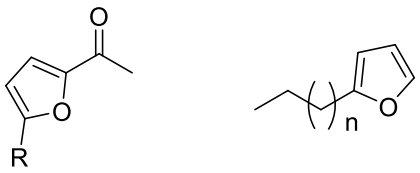
chain, fragment ion at *m/z* = 95 can be decreased into *m/z* = 82 via loss of CH^+ or splits into two fragment ions at *m/z* = 39 and 57. It can also produce fragment ion at *m/z* = 68 by the loss of CO, further releasing CH^+ group to have ion at *m/z* = 55. Therefore, the structure of the volatile **171** was suggested as 6-nonyl-2*H*-pyran-2-one.



Scheme 15 Synthesis of 6-methyl-5,6-dihydro-2*H*-pyran-2-one (**169**) and 6-nonyl-2*H*-pyran-2-one (**171**).

The reference compounds of **169** and **171** were obtained via synthesis (Scheme 15). Compound **169** was produced by esterification from 4-penten-2-ol (**173**) and acryloyl chloride (**174**) via nucleophilic addition,[368] followed by an olefin metathesis with Hoveyda-Grubbs II catalyst.[369] For the production of compound **171**, gold complex $(PPh_3)AuCl$ was used to catalyse the reaction between 1-undecene (**176**) and propiolic acid (**177**).[370] The synthetic substances, 6-methyl-5,6-dihydro-2*H*-pyran-2-one and 6-nonyl-2*H*-pyran-2-one, were proved to be identical to the volatile organic compounds (**169** and **171**), respectively, in comparison of their mass spetra and retention indices.

2.2.3 Furans



178 R = H
179 R = Me

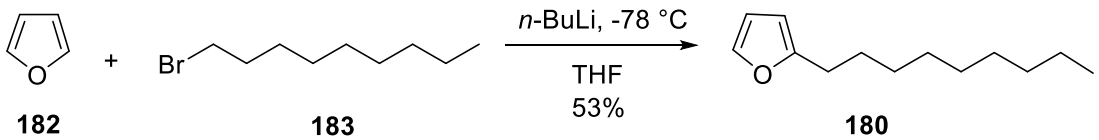
180 (n=7)
181 (n=9)

Figure 30 Furans (**178**-**181**).

Furan derivatives were also observed in the investigated fungal strains (Figure 30). 1-(2-Furanyl)-ethanone (**178**) appeared in five fungal strains, *D. clavata*, *D. childiae*, *D. hawksworthii*, *D. concentrica* and *H. macrocaipum*. Its methylated derivative **179** was produced by fungi *B. cylinderispora*. Four strains emitted 2-nonylfuran (**180**). Among them, *D. childiae* released this furan in predominate large amount. Its analogue **181** was yet only detectable in *D. childiae*.

2.2.3.1 Structural assignment of furan 180

2-Nonylfuran was obtained by the treatment of furan (**182**) with 1-bromononane (**183**) under deprotonation of *n*-butyllithium with 53% yield (Scheme 16). Synthetic **180** and natural product from fungal species showed the same mass spectrum and retention time, conforming their identity (Figure 31).



182 **183** **180**

Scheme 16 Synthesis of 2-nonylfuran (**180**).

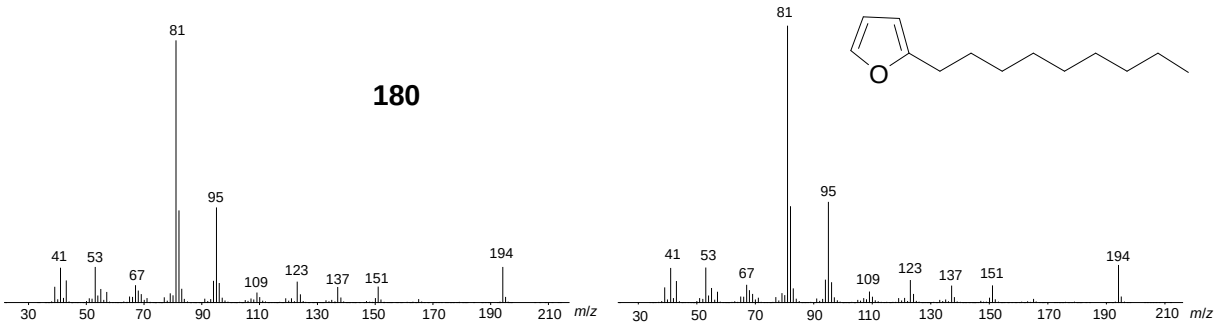


Figure 31 Mass spectra of natural product **180** (left) and 2-nonylfuran (right).

2.2.4 Nitrogen and sulfur compounds

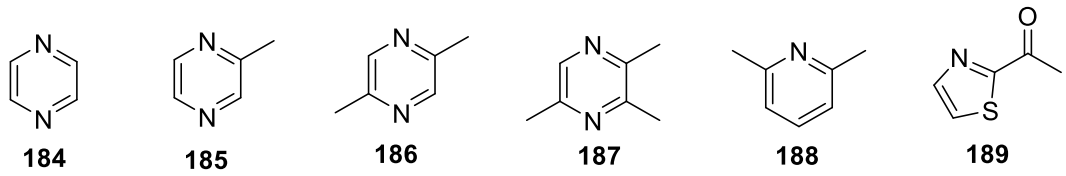


Figure 32 Nitrogen and sulfur compounds (**184**-**189**).

Several simple nitrogen and sulfur compounds were also detected in the headspace analysis (Figure 32). Pyrazine class can be easily traced in the *Daldinia* fungus. Pyrazine (**184**) was detected in *D. clavata* and *B. cylindrispora*, whereas methyl pyrazine (**185**) was found in five and 2,5-dimethylpyrazine (**186**) was produced by seven strains of 14 investigated fungus seperately. They were also previously discovered in fungistatic soils.[371] Unfortunately, trimethylpyrzine (**187**) was exclusively detectable in *H. macrocaipum*. They display the inhibitory activity against some fungal species, like *Paecilomyces lilacinus, Pochonia chlamydosporia* and *Clonostachys rosa*.[347] In previous study, the biosynthesis of the alkylated pyrazine via acetoin building blocks in *Corynebacterium glutamicum* was suggested.[347] In *D. concentrica*, 2,6-dimethylpyridine (**188**) was observed at low concentration. Finally, a thiazol derivative 2-acetylthiazole (**189**) occurred in most of all fungus.

2.2.5 Aromatic Compounds

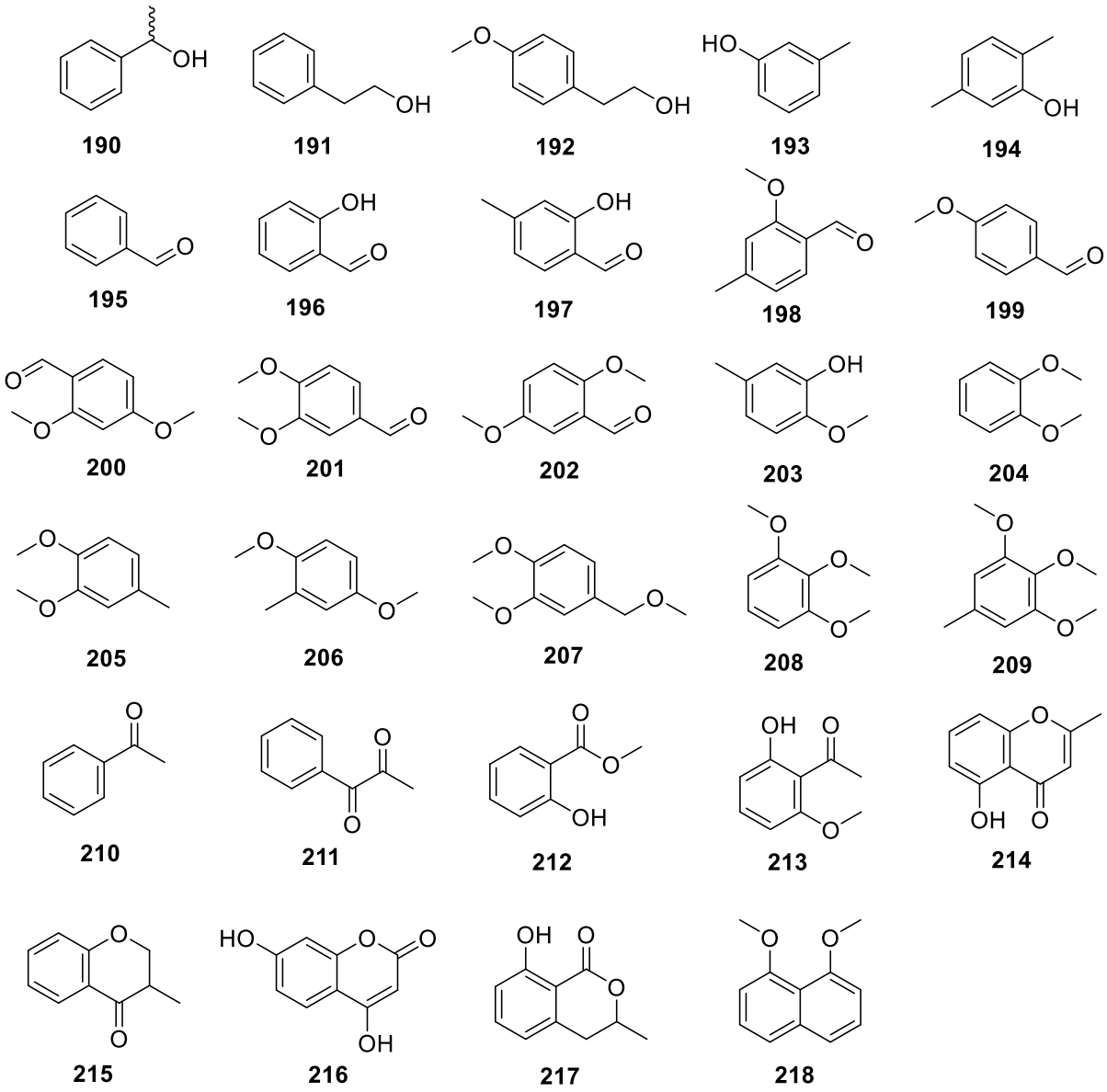


Figure 33 Aromatic compounds (**190**-**218**).

The class of aromatic compounds represents the most diverse class of secondary metabolites in the investigated fungi strains. Twenty-nine aromatic compounds were identified (Figure 33). Acetophenone (**210**) and phenylethyl alcohol (**191**) were present almost in every strain. They are very common in diverse bacteria.[279] There are two possible biosynthetic pathways to obtain **210**. On one hand, it cam start from L-phenylalanine, being transferred via cinnamoyl-CoA, attacked by water,

subsequent oxidised by a dehydrogenase and final decarboxylated.[279] On the other hand, it can begin from benzoyl-CoA, a two-carbon elongation and at last a decarboxylation.[279] As a side product in manufacturing **210**, compound **211** can be also detected in *H. macrocaipum*. Phenylethyl alcohol (**191**) is derived from a different biosynthetic pathway, arising from the same precursor, L-phenylalanine, through transamination, oxidative decarboxylation and reduction.[279]

Furthermore, α-methyl benzenemethanol (**190**) was produced as a minor product in seven fungal strains. 2-(4-Methoxyphenyl)ethanol (**192**) counld be found only in the strain *Hypoxylon* sp. nov. STMA 11183. It was reported in the extract of wood-rotting funguns *Gloeophyllum odoratum* and might be biogenetically obtained from the ligin of the host tree.[372] 3-Methylphenol (**193**) was collected from *H. macrocaipum* and was previously reported as defensive volatile in the forked fungus beetle, *Bolitotherus cornutus*.[373] However, 2,5-dimethylphenol (**194**) was present large in amount in *Hypoxylon* sp. nov. JF 11167 and *H. macrocaipum*. Its structure was also determined by direct comparison of the mass spectra and retention indices of all six dimethylphenol regiosmers obtained from commercial suppliers, thus established the identity (Table 4).

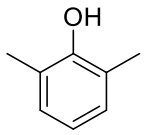
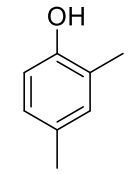
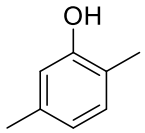
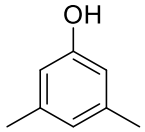
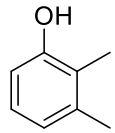
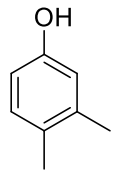
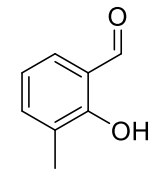
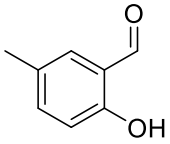
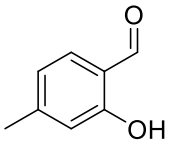
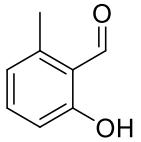
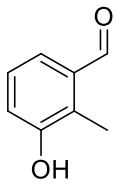
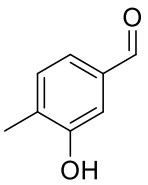
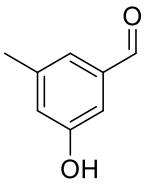
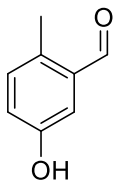
Structure	Compound name	*I* (HP-5)	MS match[a]
	2,6-Dimethylphenol	1108	862
	2,4-Dimethylphenol	1147	879
	2,5-Dimethylphenol (**194**)	1152	913
	3,5-Dimethylphenol	1164	903
	2,3-Dimethylphenol	1177	901
	3,4-Dimethylphenol	1191	892

Table 4 Retention indices of all regiosmers of dimethylphenol. [a]MS match of the mass spectrum of the natural product from fungal strain *Hypoxylon* sp. nov. JF 11167 in comparison to the mass spectrum of the tabulated compound (a value of 1000 would indicate identical mass spectra, positive compound identification can usually be assumed for a value >900).[374]

A group of benzaldehydes were also present in this fungal family. Benzaldeyde (**195**) was produced by *D. australis* and *D. concentrica*, whereas, 2-hydroxybenzaldehyde (**196**) and its ester (**212**) can be observed from *D. eschscholzii*. They are derivatives from salicylic acid which generates from chorismic acid via the shikimate pathway.[375]

Hydroxybenzaldehyde is able to improve the antifungal and anti-mycotoxigenic ability against *Aspergillus flavus* and *A. parasiticus* incorporation of other existing antifungal agents.[376]

Afterwards, hydroxy-methylbenzaldehyde compound **197** and its methoxylated derivative **198** were detected in strains *Hypoxylon* sp. nov. JF 11167 and *H. macrocaipum*. For compound **197**, all ten constitional isomers of hydroxyl-methylbenzaldehydes were pursued from chemical suppliers. A comparsion of the headspace extract from *Hypoxylon* sp. nov. JF 11167 to all these compounds by GC-MS identified **197** as 2-hydroxy-4-methylbenzaldehyde (Table 5). This compound was also isolated from Mondia whytei Skeels (*Asclepidiaceae*), which is capable of modifying taste and in charge of sweet aromatic fragrance arising from *M. whytei* root-bark.[377] On the other hand, all ten methoxy-methylbenzaldehydes were afforded by methylation of hydroxyl-methylaldehydes with the treatment of potassium carbonate and methyl iodide. The GC-MS analysis of all these compounds indicated that natural product **198** was assigned as 2-methoxy-4-methylbenzaldehyde with the same retention index and best matching mass spectrum (Table 6).

Structure	Compound name	*I* (HP-5)	MS match[a]
	2-Hydroxy-3-methylbenzaldehyde	1139	902
	2-Hydroxy-5-methylbenzaldehyde	1160	893
	2-Hydroxy-4-methylbenzaldehyde (**197**)	1165	902
	2-Hydroxy-6-methylbenzaldehyde	1202	904
	3-Hydroxy-2-methylbenzaldehyde	1374	878
	3-Hydroxy-4-methylbenzaldehyde	1396	874
	3-Hydroxy-5-methylbenzaldehyde	1399	857
	3-Hydroxy-6-methylbenzaldehyde	1408	785

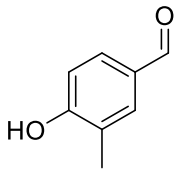
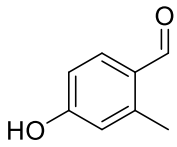
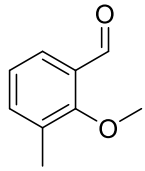
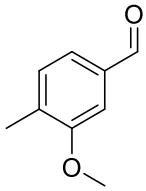
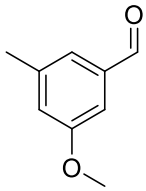
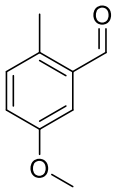
	4-Hydroxy-3-methylbenzaldehyde	1427	857
	4-Hydroxy-2-methylbenzaldehyde	1452	857

Table 5 Retention indices of all regioisomers of hydroxy-methylbenzaldehyde. [a]MS match of the mass spectrum of the natural product from fungal strain *Hypoxylon* sp. nov. JF 11167 in comparison to the mass spectrum of the tabulated compound (a value of 1000 would indicate identical mass spectra, positive compound identification can usually be assumed for a value >900).[374]

Structure	**Compound name**	***I* (HP-5)**	**MS match[a]**
	2-Methoxy-3-methylbenzaldehyde	1265	894
	3-Methoxy-4-methylbenzaldehyde	1307	697
	3-Methoxy-5-methylbenzaldehyde	1313	670
	3-Methoxy-6-methylbenzaldehyde	1323	650

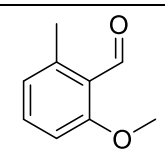
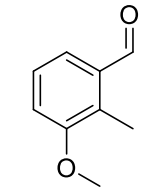
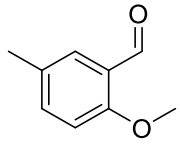
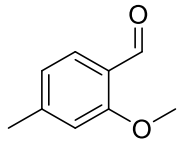
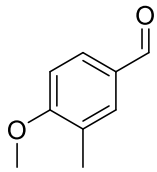
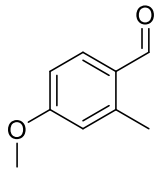
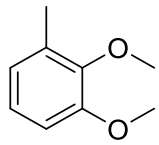
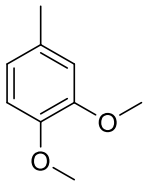
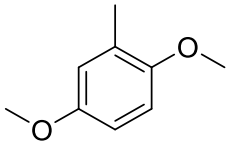
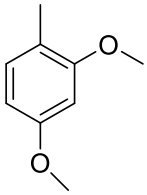
	2-Methoxy-6-methylbenzaldehyde	1335	890
	3-Methoxy-2-methylbenzaldehyde	1335	741
	2-Methoxy-5-methylbenzaldehyde	1352	884
	2-Methoxy-4-methylbenzaldehyde (**198**)	1364	934
	4-Methoxy-3-methylbenzaldehyde	1366	660
	4-Methoxy-2-methylbenzaldehyde	1368	628

Table 6 Retention indices of all regioisomers of methoxy-methylbenzaldehyde. [a]MS match of the mass spectrum of the natural product from fungal strain *Hypoxylon* sp. nov. JF 11167 in comparison to the mass spectrum of the tabulated compound (a value of 1000 would indicate identical mass spectra, positive compound identification can usually be assumed for a value >900).[374]

Besides, *D. novae-zelandiae* emitted another salicylic acid derivative, 4-methoxybenzaldehyde (**199**) in trace amount. The bastidiomycete *Polyporus sapidus* can generate and degrade this benzaldehyde quantitatively and simultaneously in terms of fast oxidation from distinct

fungal enzyme systems.[378] Three constitutional isomers of dimethoxy benzaldehydes (**200**, **201**, **202**) were present in the strains *Hypoxylon* sp. nov. STMA 11183, *H. macrocaipum* and *H. rubiginosum*, respectively. Compound **200** and **201** are likely derived from isovanillin and vanillin.[379] 2-Methoxy-5-methylphenol (**203**) was identified from *Hypoxylon* sp. nov. JF 11167, while its methylated product **205** was found in several strains, *D. novae-zelandiae*, *D. hawksworthii*, *Hypoxylon* sp. nov. JF 11167, *H. macrocaipum and H. rubiginosum*, which was also detected in Truffle *Tuber melanosporum* (Soria).[380] For unambiguous structural elucidation of **205** as a dimethoxytoluene, all six constitutional isomers were purchased from commericial suppliers and examined via GC-MS. The result showed that 3,4-dimethoxytoluene was identical for compound **205** in terms of close retention indices and the best MS match (Table 7).

Structure	**Compound name**	***I* (HP-5)**	**MS match[a]**
	2,3-Dimethoxytoluene	1176	905
	3,4-Dimethoxytoluene (**205**)	1240	937
	2,5-Dimethoxytoluene	1252	772
	2,4-Dimethoxytoluene	1257	742

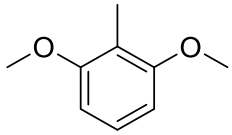
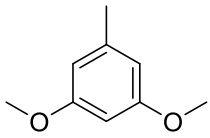
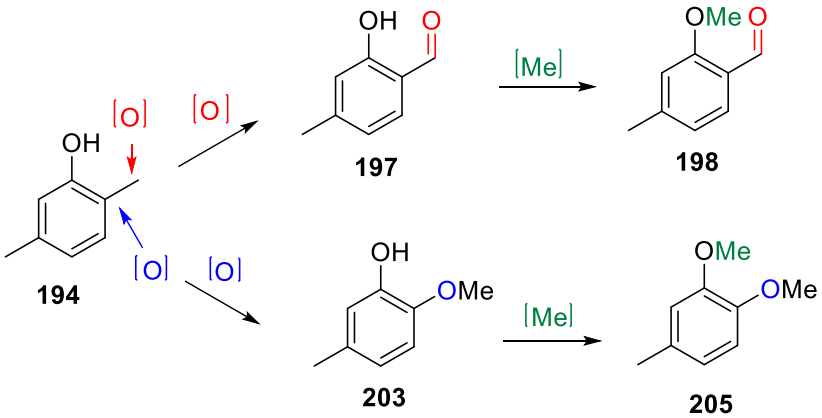
	2,6-Dimethoxytoluene	1259	779
	3,5-Dimethoxytoluene	1271	716

Table 7 Retention indices of all regiosmers of dimethoxytoluene. [a]MS match of the mass spectrum of the natural product from fungal strain *Hypoxylon* sp. nov. JF 11167 in comparison to the mass spectrum of the tabulated compound (a value of 1000 would indicate identical mass spectra, positive compound identification can usually be assumed for a value >900).[374]



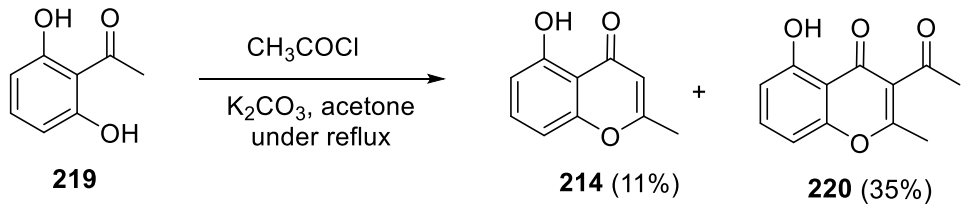
Scheme 17 Proposed biosynthesis of volatile aromatic compounds (**194**, **197**, **198**, **203** and **205**).[374]

To some structurally related aromatic compounds (**194**, **197**, **198**, **203** and **205**) released by Xylariaceae, a common biosynthesis pathway can be assumed and summarized in Scheme 17. It begins with **194**, which is either oxidised at the 2-methyl group (red) to afford the corresponding aldehyde **197**, subsequently O-methylated (green) with S-adenosylmethionine (SAM) to produce **198**, or in which a C-C bond is replaced by oxygen (blue) to result in the hypothetical intermediate **203** that is further methylated into **205** (green). This proposal could also contribute to the structural assignment of volatile compounds. For

example, compound **203** could be determined as 2-methoxy-5-methylphenol by comparsion with a commercially available standard. However, not all the regisomers with different ring substitution patterns were obtainable from chemical suppliers and other possibilities of the contitutinoal isomers cannot fully be excluded for the structural elucidation of **203**. The biosynthetic assumption described above can establish the identification of **203** as 2-methoxy-5-methylphenol from another perspective.

Deliberately, 1,2-dimethoxybenzene (**204**) occurred in *H. rubiginosum* and can be formed by the enzyme guaiacol O-methyltransferase, which methylates guaiacol.[381] 2,5-Dimethoxytoluene (**206**) and 1,2-dimethoxy-4-(methoxymethyl)benzene (**207**) were only observed in *H. rubiginosum*. Two trimethoxy-substituted components **208** and **209** were created by fungal strains *Hypoxylon* sp. nov. STMA 11183, *H. rubiginosum* and *H. macrocaipum*. Metabolite **213** was present in *D. concentrica*.

In addition, a few characteristic chromanes and naphthalene derivatives enriched the variety of this fungal family. Compound **214** is often detected in this class, for instance, *D. childiae*, *D. hawksworthii* and *Hypoxylon* sp. nov. JF 11167. Its reference compound was generated from 1-(2,6-dihydroxyphenyl)ethanone (**219**) by the treatment with acetyl chloride and potassium carbonate in acetone under reflux as a minor product (Scheme 18). In comparison of mass spectrum and retention index, the synthetic 5-hydroxy-2-methyl-4*H*-chromen-4-one proved to be the natural product **214** (Figure 34).



Scheme 18 Synthesis of 5-hydroxy-2-methyl-4*H*-chromen-4-one (**214**).

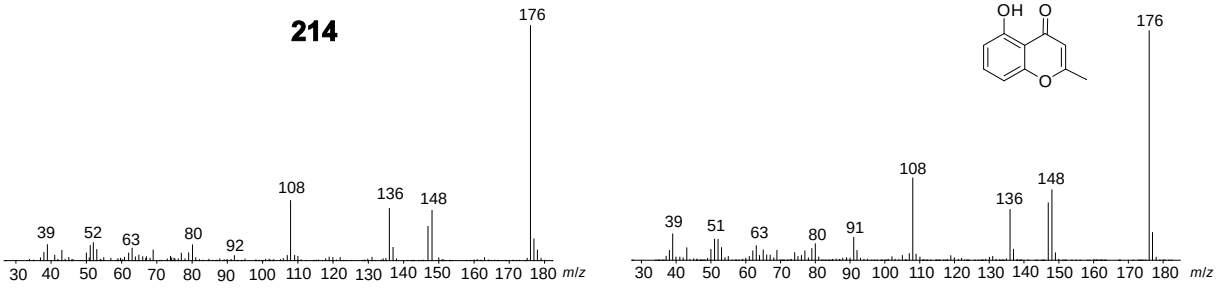


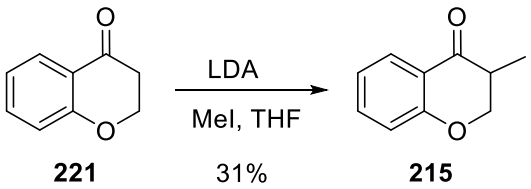
Figure 34 Mass spetra of natural product **214** (left) and 5-hydroxy-2-methyl-4*H*-chromen-4-one (right).

After that, mellein (**217**) was also found in the strain *Hypoxylon* sp. nov. STMA 11183 in small amount, which is a typical fungal metabolite initially detected in *Aspergillus melleus*[382] and also isolated from the ant *Lasius fuliginosus* as a trail pheromone.[383] Its fungicidal, antibacterial and HCV (Hepatitis-C-Virus) protease-inhibitory properties have been investigated subsequently.[384],[385] The biosynthetic gene clusters from bacteria *Saccharopolyspora erythraea* for establishing the polyketide origin of mellein has been identified.[386] The biosynthesis to this compound can be explained via a polyketide synthase, usage of acetyl-CoA as a start unit, chain extension with four malonyl-CoA units, followed by aromatic cyclization.[387]

The precursor of compound **218**, 8-methoxy-1-naphthol, has been discovered in 1960 by Allport & Bu'Lock from *D. concentrica*[388] and owns the nematicidal and antifungal activities.[389] It can be assumed that 1,8-dimethoxynaphthalene (**218**) is more likely to be derived from 8-

methyl-1-naphthol via methylation, which can be only traced in *D. childiae*. 8-Methyl-1-naphthol and 1,1'-binaphthalene-4,4'-5,5'-tetrol (BNT) are responsible for the purple dark stromatal surface of *Daldinia* species[390] and originated from pentaketide of melanin biosynthesis as side metabolic intermediates.[391]

At last, the two coumarins **215** and **216** are the main products existing in many of the analyzed fungal strains. Its reference compound, 2-methyl-4-chromanone was obtained by methylation of deprotonated chroman-4-one (**221**) with a moderate yield (31%) represented in Scheme 19. 2-Methyl-4-chromanone was conducted as natural product **215** in terms of its gas chromatographic behaviour and mass spectrum (Figure 35).



Scheme 19 Synthesis of 2-methyl-4-chromanone (**215**).

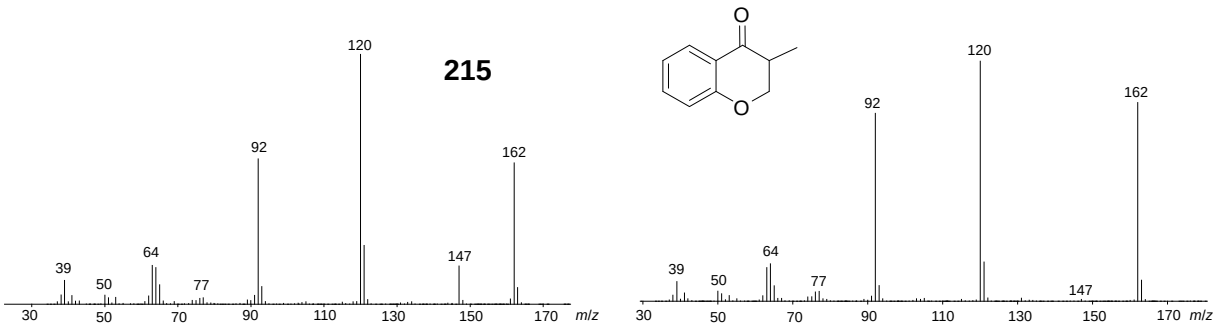


Figure 35 Mass spetra of natural product **215** (left) and 2-methyl-4-chromanone (right).

Coumarin biosynthesis has not been completely elucidated yet, although it has been an extraordinary popular target for phytochemical and pharmacological research due to its numerous bioactivities, for instance, antibacterial,[392]-[394] antioxidant,[395] anti-inflammatory,[396] rodenticidal,[397] termiticidal[398],[399] and other biological effects.[400]-[403] Biosynthesis

pathway to simple coumarins starts from phenylpropanoid pathway via *ortho*-hydroxylation (red arrows) of cinnamate (**223**) and *p*-coumarate (**226**), caffeate (**230**) and ferulate (**233**), respectively. Followed by isomerization, final lactonization can reach coumarin (**225**) and umbelliferone (**229**), esculetin (**232**) and scopoletin (**236**) (in Figure 36).[404] To understand the mechanism of simple coumarin formation, the irreversible step *ortho*-hydroxylation is extremely crucial. The identified compound **216** might be obtained via further hydroxylation in blue arrow.

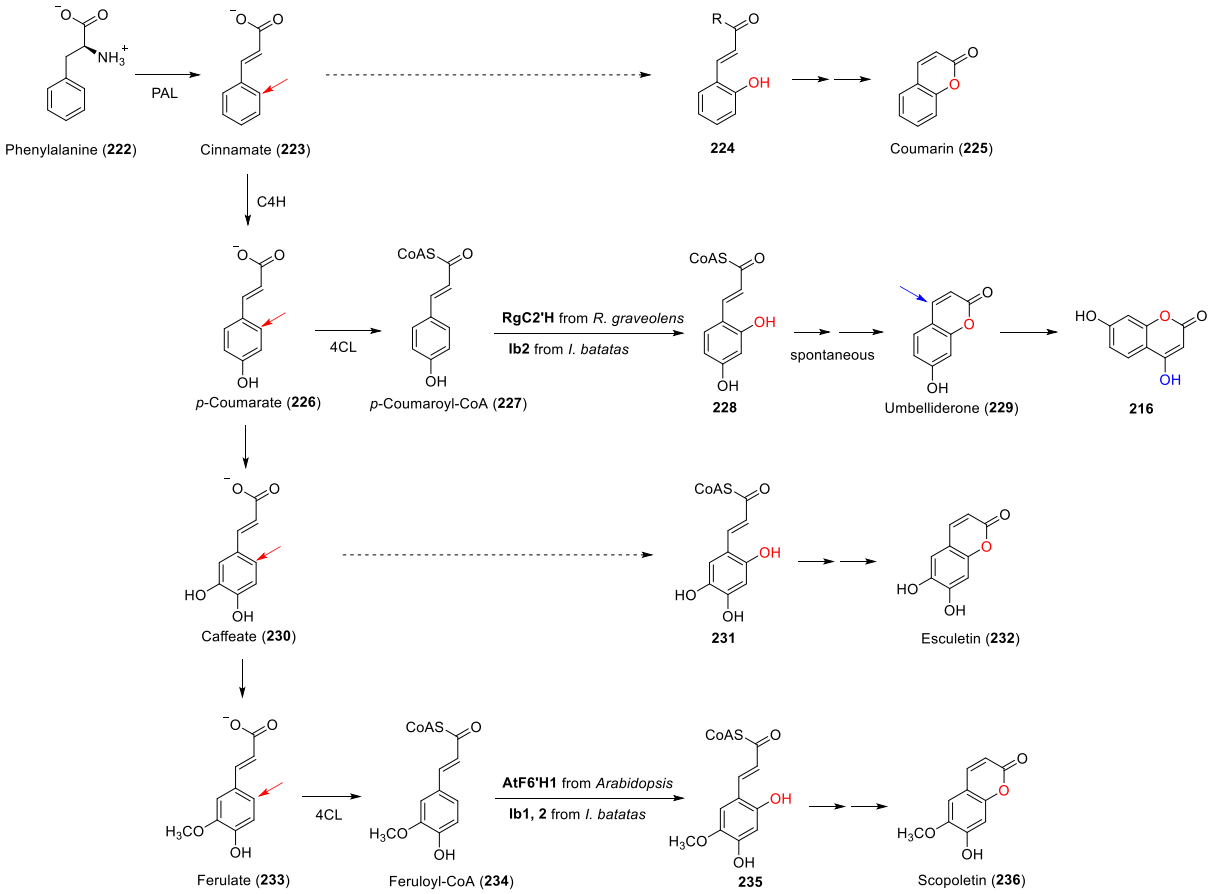
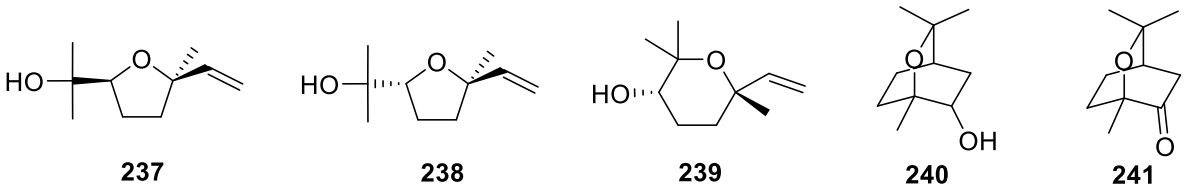


Figure 36 Coumarin biosynthetic pathway in plants. Red arrows stands for the *ortho*-positions, where oxygen atoms are introducted by *ortho*-hydroxylation. This action is performed under catalysis of the ortho-hydroxylases from Arabidopsis (AtF6'H1), *Ruta graveolens* (RgC2'H), and *Ipomoea batatas* (Ib1 and Ib2).[404] Hydroxylation in blue arrow might achieve compound **216**.

2.2.6 Terpenoids

Terpenoids constitute another prosperous class with various secondary metabolites although most of them were found in trace amounts in the investigation. Terpenoids are a rich family of natural products which can be assembled with the two building blocks dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP). The linear precusors can be generated by either mevalonate pathway or deoxyxylulose phosphate pathway.[405] Mevalonate pathway takes place in archaebacteria and most eukaryotes, including all metazoans and fungi. On the other hand, most of eubacteria, such as all Gram-negative bacteria and mycobacteria utilize deoxyxylulose phosphate pathway for the production of diverse classes of terpenoids.[406]



237 **238** **239** **240** **241**

Figure 37 Monoterpenes.

There were five monoterpenes (C_{10}), fifteen sesquiterpenes (C_{15}) and two diterpenes (C_{20}) identified in the investigated fungal strains (Figures 37 and 38). The furanoid form of *cis*-linalool oxide (**237**) and *trans*-linalool oxide (**238**) were detected in small amounts in several investigated fungal strains. For instance, in *D. clavata*, *D. childiae*, *D. novae-zelandiae*, *H. rubiginosum* and *B. cylinderispora*. Both compounds were isolated as major aglycone moieties in fresh tea leaves and exhibited considerable antifungal activities against *Colletotrichum camelliae* Massea.[407] The pyranoid form of *cis*-linalool oxide (**239**) was demonstrated in the headspace extracts of *D. clavata*, *D. childiae* and *B. cylinderispora*.

Another two monoterpenes, 2-hydroxy-1,8-cineole (**240**) and 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-6-one (**241**), showed very high amounts in the extracted volatile mixture of *H. griseobrunneum*. In Omarini's work, both substrates were considered as the oxygenated outcomes by transformation of 1,8-cineole through solid-state fermentation (SSF) with two mushrooms *Pleurotus ostreatus* and *Favolus tenuiculus*.[408] 1,8-Cineole was reported as *eucalyptol* after discovered by Cloez in 1870 as major product in *Eucalyptus globulus* plant oil.[409] Recently, a series of VOCs from three endophytic fungi species of the family Xylariaceae, *Hypoxylon* sp. and related anamorph *Nodulisporium* sp. were able to generate 1,8-cineole.[410]-[412] Meanwhile the identification of the *hyp*3 gene expressed in *Escherichia coli*, the first monoterpene synthase set the milestone for fully understanding the 1,8-cineole biosynthesis, thereby supplied us with a new perspective of differing terpene synthases in fungi in comparison to those in plants and bacteria.[405]

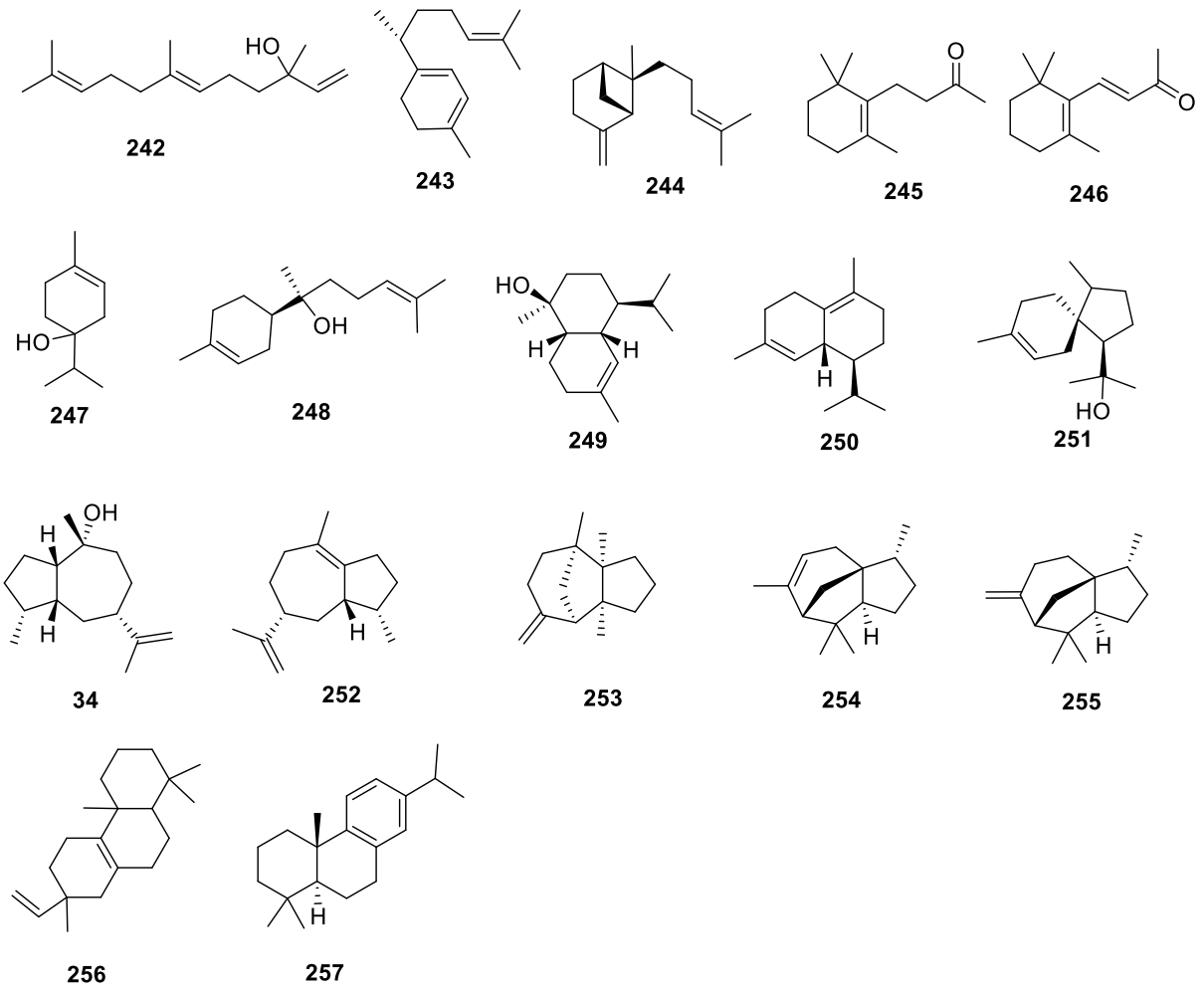


Figure 38 Sesquiterpenes and diterpenes.

For the sesquiterpenes, most of them were present in specific fungal strains, beside pogostol (**34**) and α-bulnesene (**252**). Pogostol (**34**) is a major product in *D. australis*, *D. cf. caldarium and D. concentrica*. But it is present in a small scale in *D. clavata*, *D. eschscholzii*, *D. hawksworthii* and *B. cylinderispora*. It is one of the extracts in musky oil from the patchouli plant and has been utilized in traditional Chinese medicine due to its antiemetic properties.[413] Weyerstahl completed the full structural assignment of pogostol by NMR.[414] In previous study, the pogostol biosynthesis was suggested by initial feeding experiments with six ^{13}C-labelled isotopomers of mevalonalactone in fungus *Genuculosporium* and subsequent analysis of its secondary metabolites with CLSA-NMR.[284] *D. australis*, *D. cf. caldarium* and *D. concentrica* emitted α-

bulnesene (**252**) in trace amount. It was also extracted from *Pogostemon cablin* and can restrain platelet-activating factor (PAF) and arachidonic acid (AA) in order to reduce rabbit platelet aggregation.[415]

Besides that, the rest thirteen sesquiterpenes were located differentially in certain fungal species. *D. clavata* emitted γ-curcumene (**243**), which is furnished from (2*E*,6*E*)-farnesyl diphosphate under the catalysis of γ-curcumene synthase.[416] *trans*-β-Bergamotene (**244**), described as the first male pheromone in the parasitic Hymenoptera.[417] The gene cluster of fumagillin biosynthesis in *Aspergillus fumigatus* was responsible for the production of this terpene.[418] *trans*-β-Bergamotene (**244**) can only be found in *D. childiae*, where nerolidol (**242**) was also detected.

In *D. concentrica*, dihydro-β-ionone (**245**) and its analogue *trans*-β-ionone (**246**) were detected. They are widely present in different flowers and fruits, for instance, blackberries, peaches and apricots,[419] and used extensively in the flavoring industry due to their characteristic aromatic scent.[420] Terpinene-4-ol (**247**) was generated by fungal species *Hypoxylon* sp. nov. JF 11167. Its antifungal activity in vivo was tested against mycotoxigenic plant pathogens.[421] *D. australis* provided T-muurolol (**249**), which was previously isolated from *Calocedrus macrolepis* var. formosana Florin leaf and showed strongly inhibition against the growth of *Rhizoctonia solani* and *Fusarium oxysporum*.[422] α-Bisabolol (**248**) and α-acorenol (**251**) were found in *H. rubiginosum*. *Biscogniauxia cylinderispora* was enabled to create δ-cadinene (**250**). β-Barbatene (**253**) was released by *D. australis* and its biosynthesis is fully understood by using ^{13}C- and ^{2}H-labelled acetate, mevalonate and glycerol.[423]

In addition, two isomers, α-cedrene (**254**) and β-cedrene (**255**) were released by *H. macrocaipum*. The biosynthesis of these two sesquiterpenes were elucidated and determined by analysis of volatile compounds from the fungus *Fusarium verticillioides.*[424]

Ultimately, two diterpenes were also identified in different strains. 8(14),15-Pimaradiene (**256**) was found in *D. hawksworthii*, and abietatriene (**257**) was detected in the volatiles of and *B. cylinderispora* respectively.

The results of this project lead to conclude that 88 diverse fungal volatiles from 14 fungal strains of the fungal Xylariaceae family were discovered, including fatty acids derivatives, lactones, furans, nitrogen and sulfur compounds, aromatic compounds, and terpenes. The structures for these metabolites have been suggested based on their mass spectra and on a retention index increment system. Several certain volatiles were approached and verified by the synthesis of key reference compounds or commercial suppliers.

2.3 Oxazolidinone derivatives

2.3.1 Synthesis of linezolid derivatives

As described in the introduction chapter, linezolid derivative **92** was considered as a potential antimicrobial agent owing to its low enthalpic energy in binding affinity to the ribosomel model. Therefore, the stereoselective total synthesis to the targe molecule was performed and its isomer **258** was yielded through the similar strategy as well.

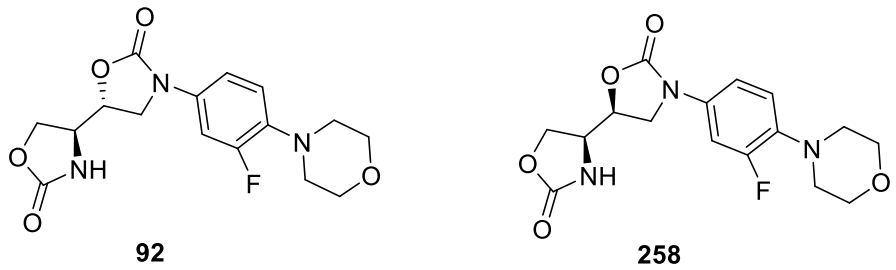
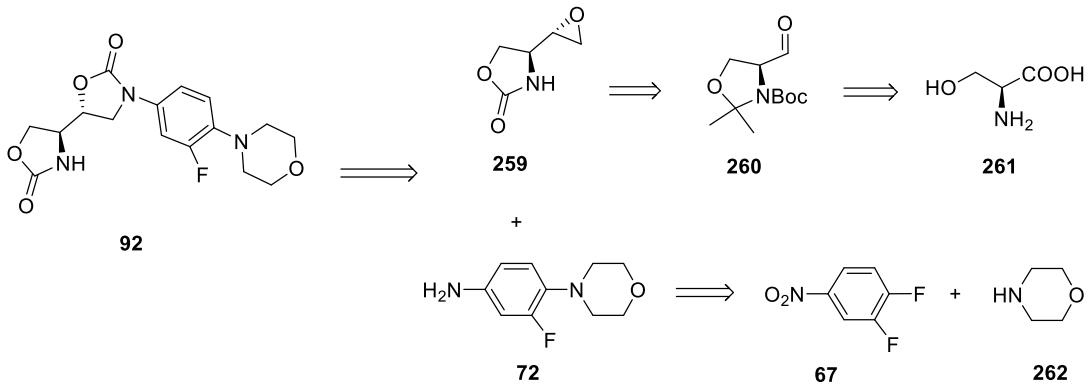


Figure 39 Linezolid derivatives **92** and **258**.

2.3.1.1 Retrosynthesis of (4*S*,5'*R*)-3'-(3-fluoro-4-morpholinophenyl)-[4,5'-bioxazolidine]-2,2'-dione (92)

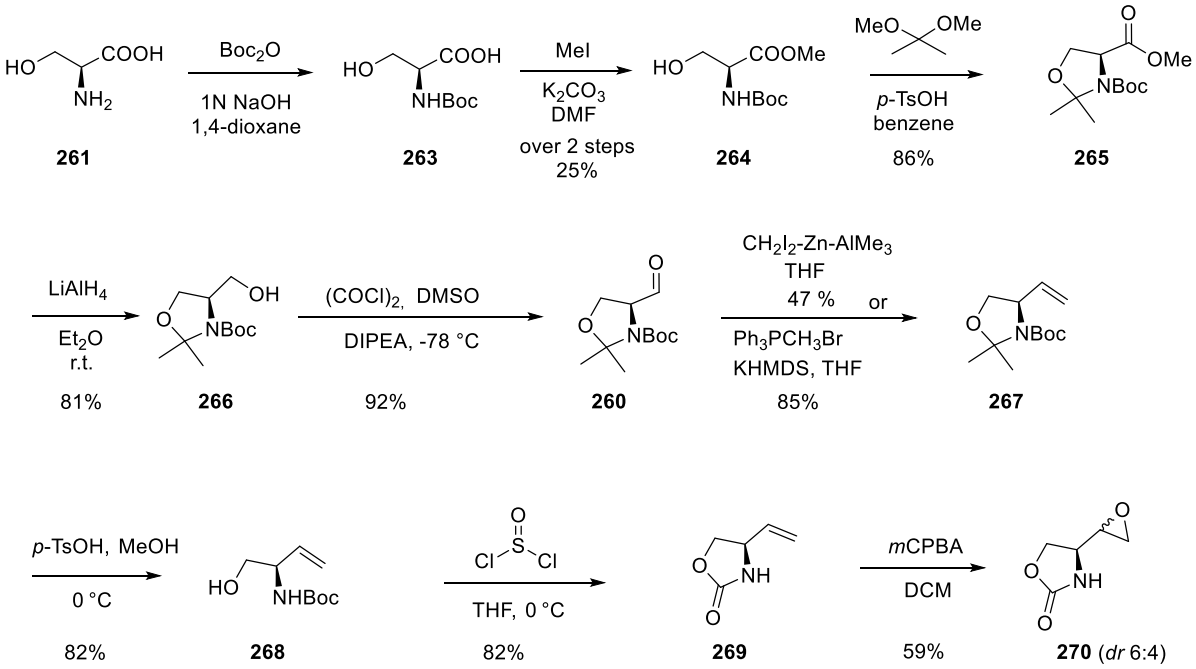


Scheme 20 Retrosynthetic analysis to oxazolidinone **92**.

As shown in Scheme 20, target compound can be reached by nucleophilic attack from amine **72** at the epoxide **259**. This amide can be derived from morpholine **262** and 3,4-difluoronitrobenzene (**67**). Meanwhile, the epoxide can be obtained from Garners' aldehyde **260**. In

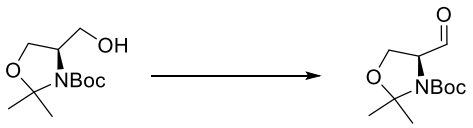
literatures, several protocols have described the stereoselective conversion from L-serine **261** to this aldehyde.[425]

2.3.1.2 Synthesis of the epoxide 270



Scheme 21 Synthetic Pathway to epoxide **270**.

The total synthesis of molecule **92** can be divided into two parts. For the synthesis of epoxide **270**, it initially began with L-serine (**261**) by protection of di-*tert*-butyl dicarbonate under basic condition in 1,4-dioxane to reach the Boc-protected amide **263**.[426] Subsequently, methylation in carboxylic group with usage of methyl iodide and potassium carbonate in dimethylformamide generated compound **264**.[426] The moderate yield over two steps was 25%. It was then treated with 2,2-dimethoxypropane and *para*-toluenesulfonic acid in hot benzene resulting in the acetonide protected serine ester **265** efficiently with 86% conversion.[426] Reduction with lithium aluminium hydride was carried out to introduce alcohol **266** in a reasonable yield of 81%.[427]



266 260

Entry	Reagents	Solvents	Reaction Condition	Yield (%)	Optical Activity ([α] 20/D)
1	DMP	DCM	r.t.	72	-70.88°
2	$(COCl)_2$, DMSO, DIEPA	DCM	-78 °C	92	-77.30°
3	TEMPO, NaOCl, NaBr, $NaHCO_3$	toluene/EtOAc/H_2O (6:6:1)	0 °C	65	-87.28°
					-90° (Lit.)

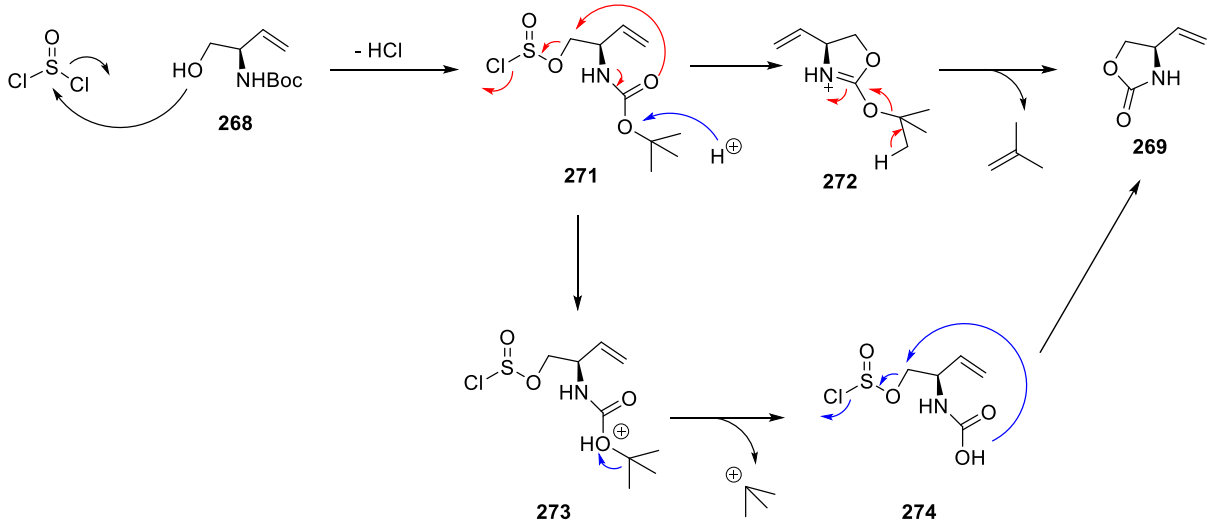
Table 8 Different oxidation methods in the production of aldehyde **260**.

For having Garner's aldehyde **260**, three distinct oxidation approaches were implemented (detailed in Table 8). Among them, Dess-Martin periodinane (DMP) oxidation demands the most simple reaction condition[428] but preparation of DMP reagent in traditional routine can be toxic and dangerous owing to its byproduct, iodine.[429] Optical activity of the product also has the largest difference compared to value in literature.[426] It can be resulted from the inpurity or further oxidation into the corresponding carboxylic acid. Swern oxidation with oxalyl chloride, DMSO and *N*,*N*-diisopropylethylamine at -78 °C led to Garner's aldehyde (**260**) with an excellent yield (92%).[430] TEMPO oxidation with sodium hypochlorite, sodium bromide and sodium bicarbonate in cold solvent mixture of toluene, ethyl acetate and distilled water (6:6:1) generated **260** with the least epimerization of α-center in an acceptable yield (65%).[431]

Afterwards, olefination with the zinc/methylene iodide/trimethylaluminum reagent gave optical pure material in an acceptable yield (43%).[432] Optimal method under salt-free Wittig conditions using methyltriphenylphosphonium bromide (Ph_3PCH_3Br) and potassium

bis(trimethylsilyl)amide (KHMDS) as base produced **267** in excellent yield and high optical purity (85%).[433]

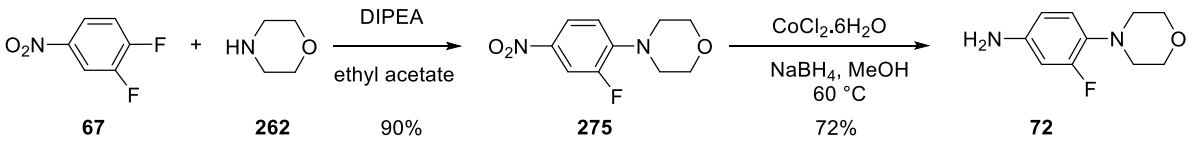
Later, the acetonide protecting group was cleavaged by *para*-toluenesulfonic acid in cold methanol and resulted in **268** in a good yield (82%).[434] First oxazolidinone ring was cyclized by the addition of thionyl chloride in cold tetrahydrofuran yielding olefine **269** (82%).[435] Two possible mechanisms of this process were depicted in Scheme 22. Initially, the hydroxyl group in thionyl chloride attacks upon thionyl chloride and loses hydrochloride. Subsequently, nucleophilic attack from the carbonyl group in carbamate moiety to α-carbon generates the oxazolidinone ring **274**. This produces an amine cation and cleavages sulfur dioxide. Due to the deprotonation of the *tert*-butyl group, compound **269** can be achieved (in red arrows). Another possibility is that the electron moves into the oxygen atom so that *tert*-butyl group is cleavaged and carboxyl acid is formed, which allows a S_N 1 reaction at α-carbon to obtain the oxazolidinone ring and split sulfur dioxide (in blue arrows).



Scheme 22 Two possible mechanisms (red and blue arrows) of formation of oxazolidinone **269**.

Last step was the epoxidation of olefin **269** with *meta*-chloroperoxybenzoic acid, which produced a mixture of diastereomers, **270** (*dr* 6:4) with an acceptable yield (59%).

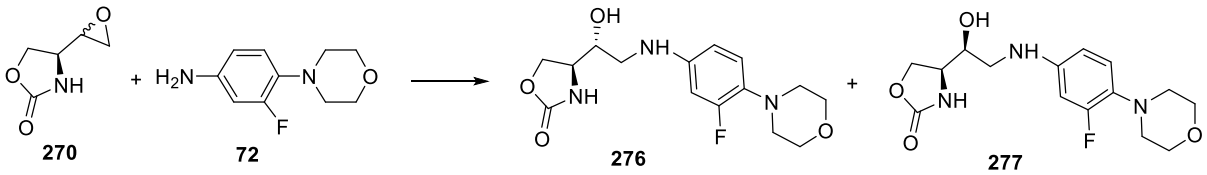
2.3.1.3 Synthesis of 3-fluoro-4-morpholinoaniline (72)



Scheme 23 Synthesis of 3-fluoro-4-morpholinoaniline **72**.

On the other hand, morpholine derivative **72** was synthesized from 3,4-difluoronitrobenzene (**67**) and excess morpholine (**262**), via a nuclephilic aromatic displacement to selectively obtain the *p*-substituted nitrobenzene **275** in an excellent conversion (92%).[319] This was later reduced by excess sodium borohydride and cobaltous chloride in warm methanol with a moderate yield (72%).[435]

2.3.1.4 Nucleophilic attack from amine 72 to epoxide 270



Entry	Catalysts	Solvents	Reaction conditions	Yield (%)
1	LiOTf	MeCN	60 °C	0
2	LiBr	THF	r.t.	0
3	$SnCl_4 \cdot 5H_2O$	THF	50 °C	0
4	$NiCl_2 \cdot 6H_2O$	THF	50 °C	0
5	$ZnCl_2$	THF	50 °C	0
6	CuI	THF	50 °C	0
7	$ZrCl_4$	THF	r.t.	0
8	$CoCl_2$	MeCN	r.t.	0
9		DMF	70-80 °C	0
10		EtOH/H_2O (9:1)		0

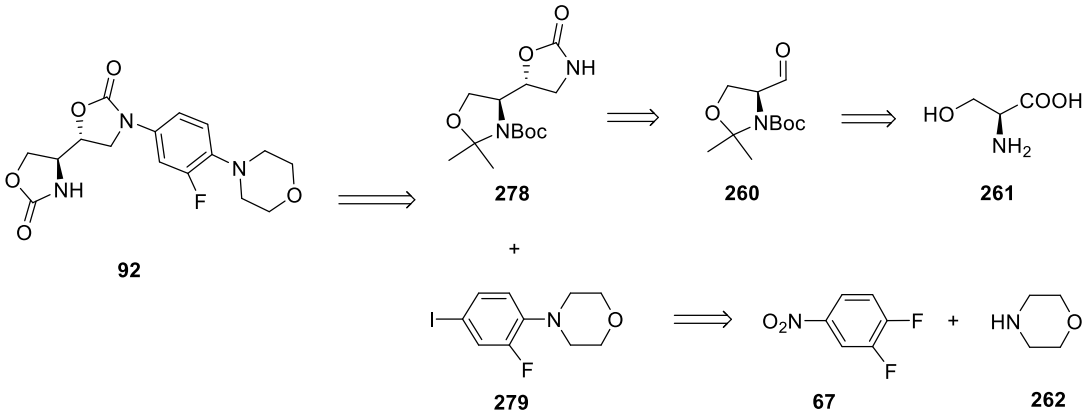
Table 9 Testing reaction conditions for epoxide ring opening with amines.

The following key step was the opening of the epoxide ring with a nucleophilic attack from amine **72**. Therefore, a variety of distinct reagents, catalysts, reaction solvents, and reaction conditions have been evaluated in Table 9. For instance, amine **72** was tried to open the terminal epoxide in warmed acetonitrile solution of lithium trifluoromethanesulfonate by internal S_N2 reaction.[436] However, no conversion was observed. Thus, the activation of the epoxide was further investigated. For Li^+ owns a strong oxophilicity, which enables to activate oxygen-containing electrophiles for nucleophilic attack,[437],[438] thence lithium bromide was used to catalyse the opening of epoxide rings by amines.[439] Besides, a group of transition metal-based lewis acids were also evaluated, including tin(IV) chloride, nickel chloride, zinc chloride, copper(I) iodide.[440] Due to the efficient catalytic performance in

nucleophilic epoxide ring opening by amines, zirconium(IV) chloride[441] and cobalt(II) chloride[442] were attempted as well. Further trials with different solvents, for example dimethylformamide[443] or mixture of ethanol and distilled water (9:1),[444] were additionally investigated. Unfortunately, neither of them was succeeded in the combination of the two fragments.

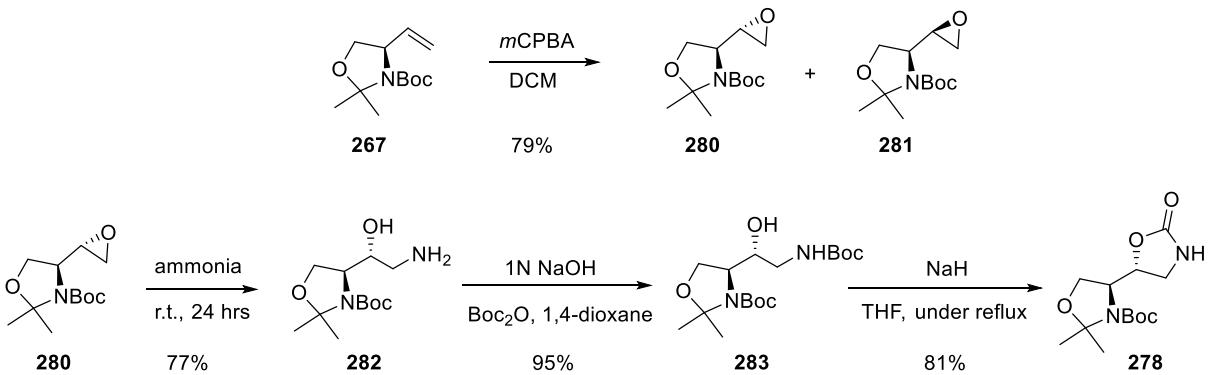
2.3.1.5 Adjusted retrosynthetic analysis to oxazolidinone 92

In consequence, the retrosynthetic plan was changed (Scheme 24). Through a Buchwald-Hartwig amination,[445] molecule **92** can be derived from coupling of **278** and **279**.



Scheme 24 Adjusted retrosynthetic analysis to oxazolidinone **92**.

2.3.1.6 Synthesis of oxazolidinone 278



Scheme 25 Synthesis of oxazolidinone **278**.

As shown in Scheme 25, slight alterations in synthetic approach took place in order to afford target molecule. Vinyl oxazolidine **267** was converted into epoxide with *meta*-chloroperoxybenzoic acid in an acceptable yield (79%). With repeated column chromatography purifications, the epoxides **280** and **281** were separated from each other in a ratio of 2.2:1. The crystal structure of **281** was determined by X-ray crystallography (Figure 40). Then, the desired major compound **280** was treated with aqueous ammonia and converted into the corresponding amino alcohol **282** efficiently (77%).[446]

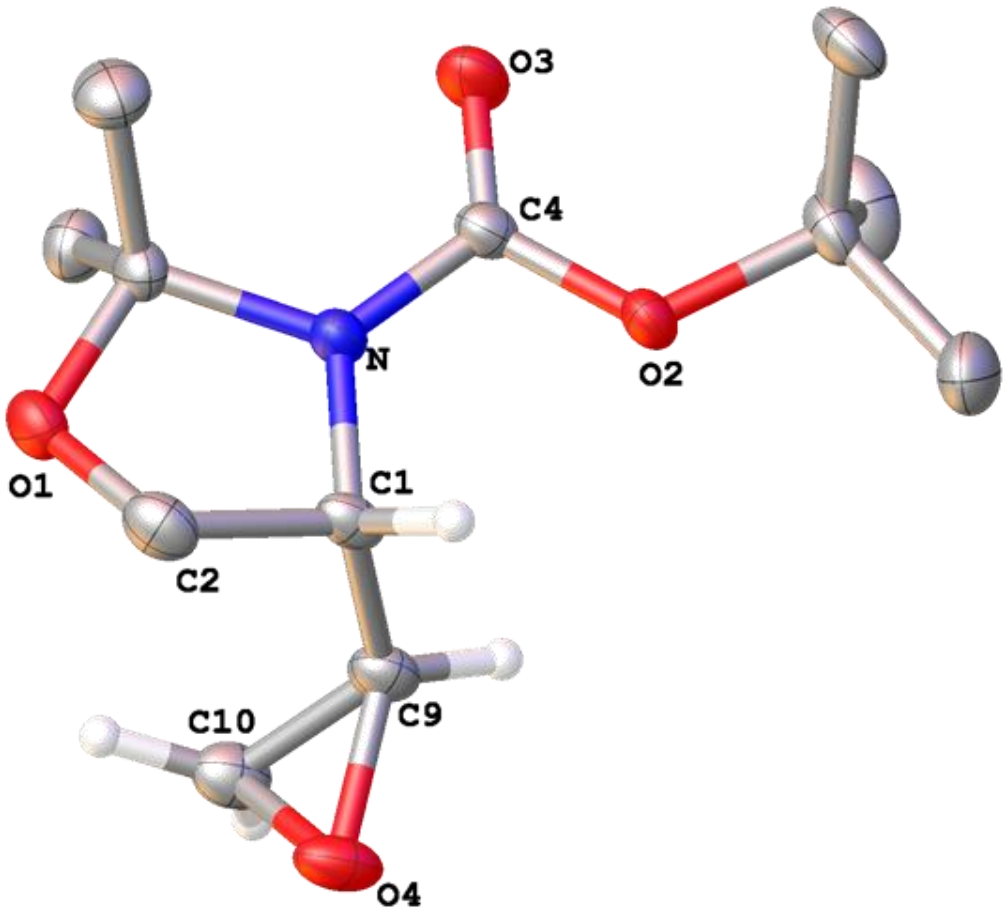
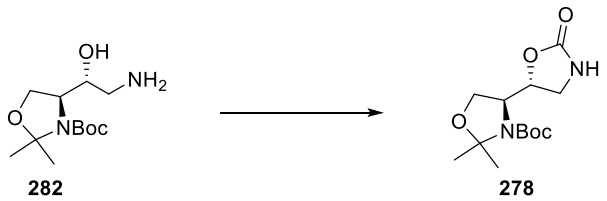


Figure 40 The ORTEP crystal structure of epoxide **281**. Grey colour stands for carbon, white represents hydrogen atoms, oxygen atoms are painted in red and blue depicts nitrogen.

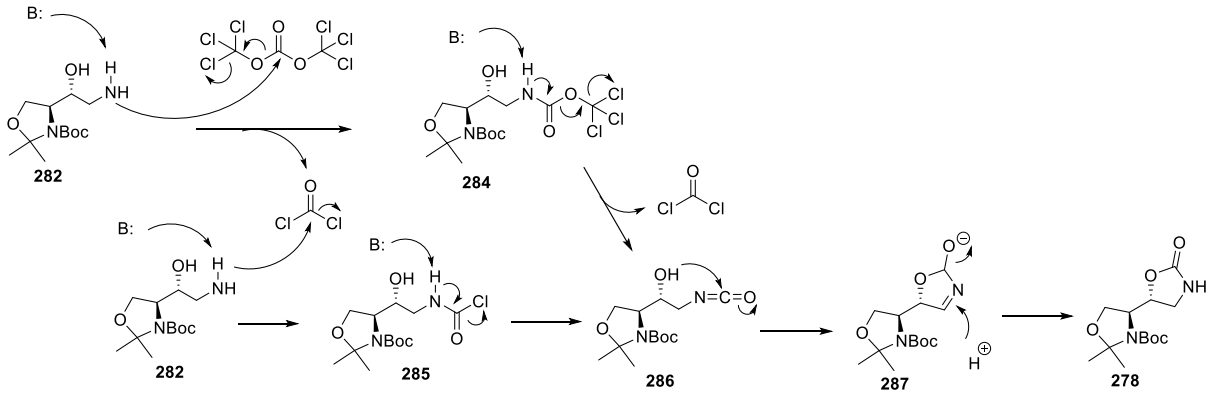


282 278

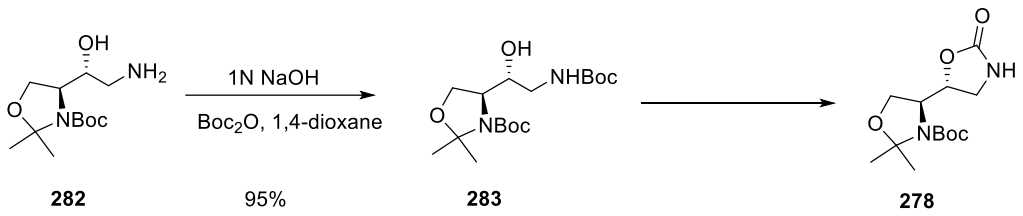
Entry	Reagents	Solvents	Reaction Conditions	Yield (%)
1	*N*,*N'*-carbonyldiimidazole	THF	r.t., 20 hrs	0
2	*N*,*N'*-carbonyldiimidazole, TEA	DCM	r.t., 20 hrs	0
3	diethyl carbonate, K_2CO_3		under reflux	0
4	triphosgene, TEA	DCM	0 °C	42
5	triphosgene, DIEPA	DCM	0 °C	31
6	triphosgene	THF	under reflux	14

Table 10 Testing reaction conditions for formation of compound **278**.

Several reaction conditions were considered in the purpose of the construction of 1,3-oxazolidin-2-one **278** (described in Table 10). α-Amino alcohol **282** was treated with *N*,*N'*-carbonyldiimidazole either in anhydrous tetrahydrofuran[447] or under triethylamine basic condition in dry dichloromethane[436] through an intramolecular attack of the primary amine function at the carbonyl group of the carbamate moiety in order to afford compound **278**. The reactions were stirred at room temperature over 20 hours, however, starting material was entirely recovered and no product was observed. In another case, diethyl carbonate and potassium carbonate as base were added and stirred under reflux,[448] but no product was found. Besides, alternative solutions with employment of triphosgene was added into the reaction mixture with triethylamine[449] or Hünig's base[450] in cold dichloromethane, or to THF and heated under reflux for 1 hour.[451] All of them succeeded in introducing the oxazolidinone auxiliary **278**. The mechanism is depicted in Scheme 26. Nucleophilic attack of the amine group in **282** to triphosgene brings isocyanate **286** via **284** or **285**, followed by cyclization and protonation of anion **287** to afford **278**.



Scheme 26 Proposed mechanism in the production of **278** with triphosgene.



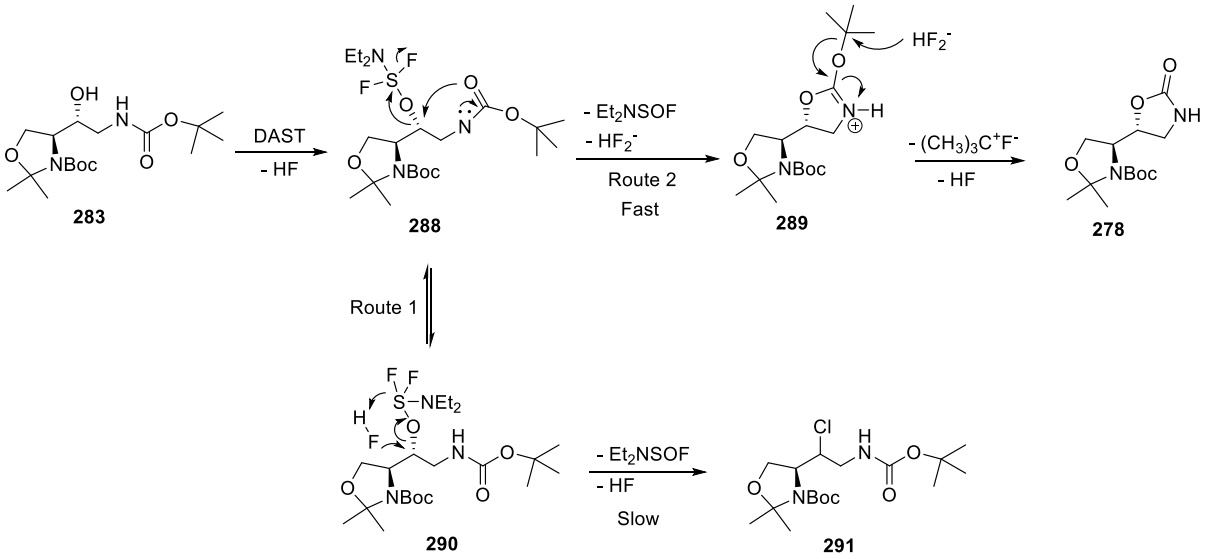
282 95% **283** **278**

Entry	Reagents	Solvents	Reaction Conditions	Yield (%)
1	thionyl chloride	THF	0 °C	0
2	DAST	THF	0 °C	0
3	Ph_3P-CCl_4-Et_3N	MeCN	r.t.	0
4	Ph_3PCl_2	DCM	0 °C	0
5	MsCl, DIPEA	THF	0 °C	0
6	TsCl	pyridine	60 °C	0
7	^{t}BuOK	THF	0 °C	0
8	NaH	THF	under reflux	81

Table 11 Synthesis of oxazolidinone **278** via intermediate **283**.

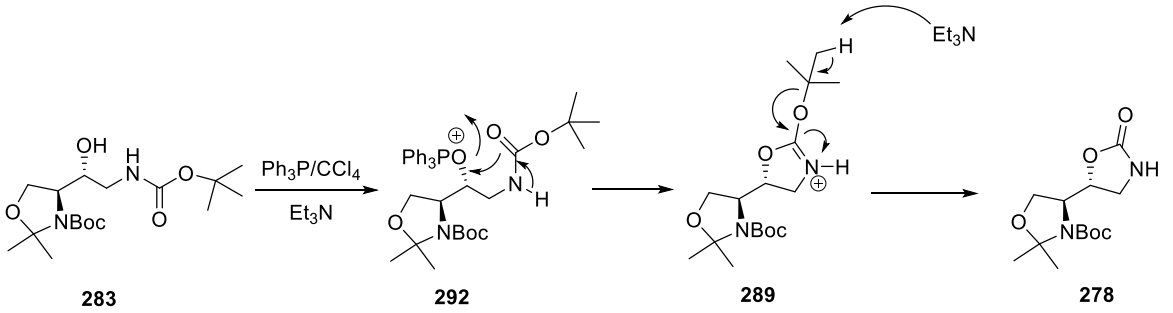
In addition, Boc-protection under basic condition in 1,4-dioxane was carried out and produced compound **283** quantitatively (95%). The ring closure was performed with several reagents (Table 11). Thionyl chloride in cold tetrahydrofuran was tried again but failed.[434] Theoretically, treatment with *N*,*N*-diethylaminosulfur trifluoride (DAST) under mild condition can lead to compound **283** via an intramolecular cyclization.[452] As stated in Scheme 27, Boc-protected amino alcohol **283** reacts with DAST to afford the intermediate **288**, then either via a regular fluorinating

mechanism further developing the fluorinated compound **291** (Route 1) or forming a more stable five member cationic intermediate **289** by an intramolecular S_N2 mechanism, subsequently to lose the *tert*-butyl group and yield the heterocycle **278** (Route 2).[452] Unfortunately, this approach did not work out either.



Scheme 27 Proposed mechanism of cyclisation of **283** with DAST.[452]

Another possibility is the usage of triphenylphosphine-carbon tetrachloride-triethylamine (PPh_3-CCl_4-Et_3N) system, via an S_N2 process, nucleophilic attack of the carbonate on the activated hydroxyl bearing center. This shoud lead to cyclization and formation of the desired product (Scheme 28).[453] However, this reagent failed and no product was examined. The alternative reagent, dichlorotriphenylphosphorane was also attempted but no product was detected.[432]

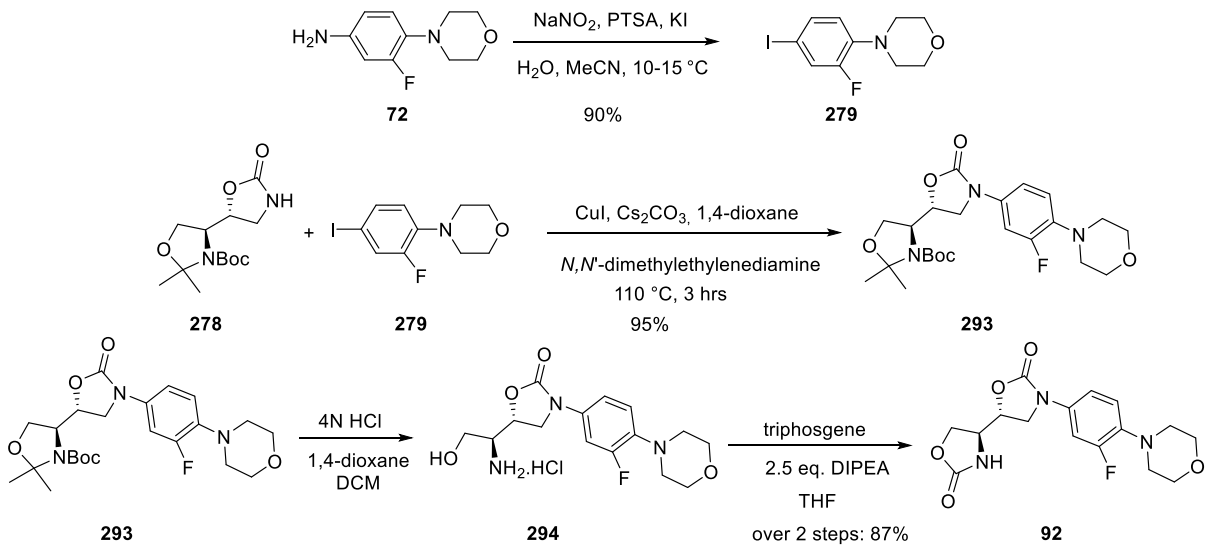


Scheme 28 Proposed mechanism of cyclisation of **283** with Ph_3P-CCl_4-Et_3N system.

Furthermore, mesylation and tosylation were taken in consideration as well. Thereby, a nucleophilic displacement of the mesyloxy and tosyloxy leaving group by the carbamate moiety drives the formation of oxazolidinone ring.[454] The former method exploited methanesulfonyl chloride and *N*, *N*-diisopropylethylamine (DIPEA) in cold dichloromethane[454] and the inferior implanted *para*-toluenesulfonyl chloride in warm pyridine. Unfortunately, neither of them completed the cyclisation.

Base catalysed cyclisation with potassium *t*-butoxide in anhydrous tetrahydronfuran via an alkoxide species as intermediate was likewise performed in this study.[455],[456] Finally, compound **283** was treated with sodium hydride in hot tetrahydrofuran to form first oxazolidinone auxiliary **278** in a good yield (81%).[457]

2.3.1.7 Formation of oxazolidinone 92

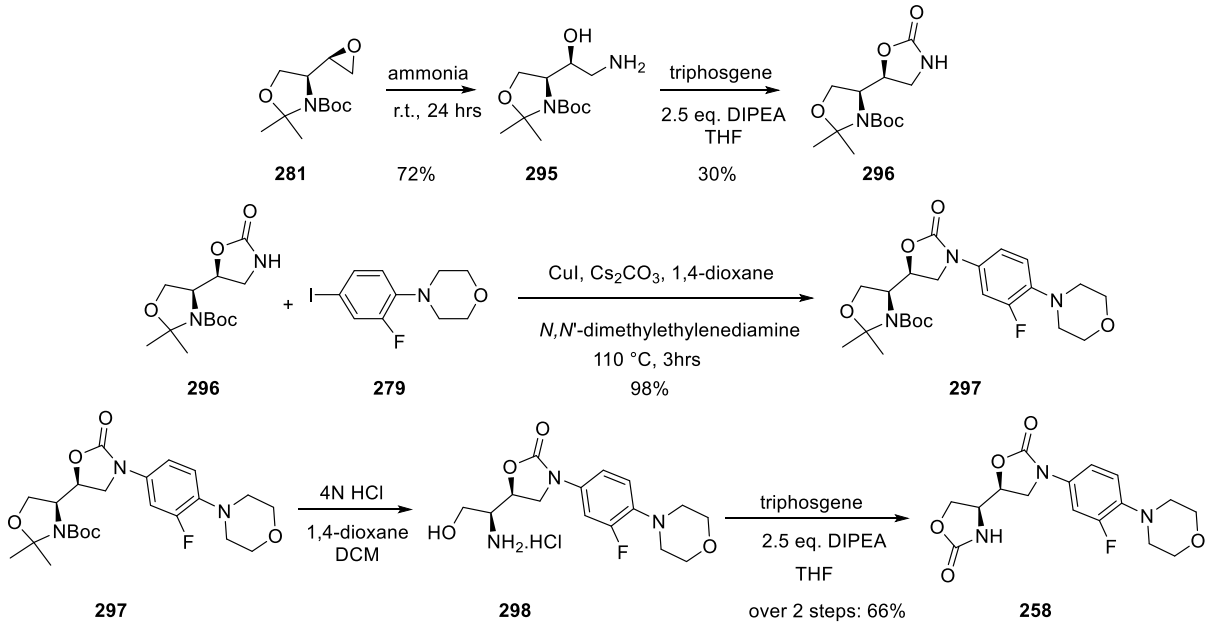


Scheme 29 Synthesis of oxazolidinone **92**.

Furthermore, diazotization of amine **72** with sodium nitrite and *para*-toluenesulfonic acid, followed by iodination with potassium iodide resulted in iodo compound **279** with 90% conversion.[445],[458] The

significant transformation in this entire synthesis was the transition metal catalysed C-N bonding-forming processes. Herein, according to Buchwald protocol,[459],[460] copper iodide was employed as catalyst, caesium carbonate as base and *N*,*N'*-dimethylethylenediamine as ligands so that the coupling between oxazolidinone **278** and iodo component **279** was made to afford compound **293** in excellent yield (95%).[445] The last step was the cleavage of both protecting groups, under strong acidic condition (4N hydrochloric acid) and the treatment with excess *N*,*N*-diisopropylethylamine and triphosgene in cold tetrahydrofuran to introduce the second oxazolidinone ring **92** (87% over two steps).[461] The overall yield of the longest linear sequence was 5% in 13 steps.

2.3.1.8 Synthesis of oxazolidinone 258



Scheme 30 Synthesis of oxazolidinone **258**.

The same strategy was applied for the production of another oxazolidinone derivative **258**. Epoxide **281** was attacked by ammonia and converted into the corresponding amino alcohol **295** (72%),[446]

followed by ring closure by treatment with triphosgene and *N*,*N*-diisopropylethylamine to achieve oxazolidinone **296** in moderate yield (30%).[461] Cross coupling reaction under Buchwald conditions generated **297** efficiently (98%).[445] Under strong acidic condition, the protecting groups were cleavaged and the treatment with triphosgene and excess *N*,*N*-diisopropylethylamine introduced another oxazolidinone ring in **258** (66% yield over two steps).[461] The overall yield of the longest linear sequence was 1.6% in 12 steps. The crystal structures of both linezolid derivatives **92** and **258** were show in Figure 41. These also confirmed the stereochemical question in the production of both compounds containing two distinct stereocenters. Their biological evaluation will be discussed in the following chapter.

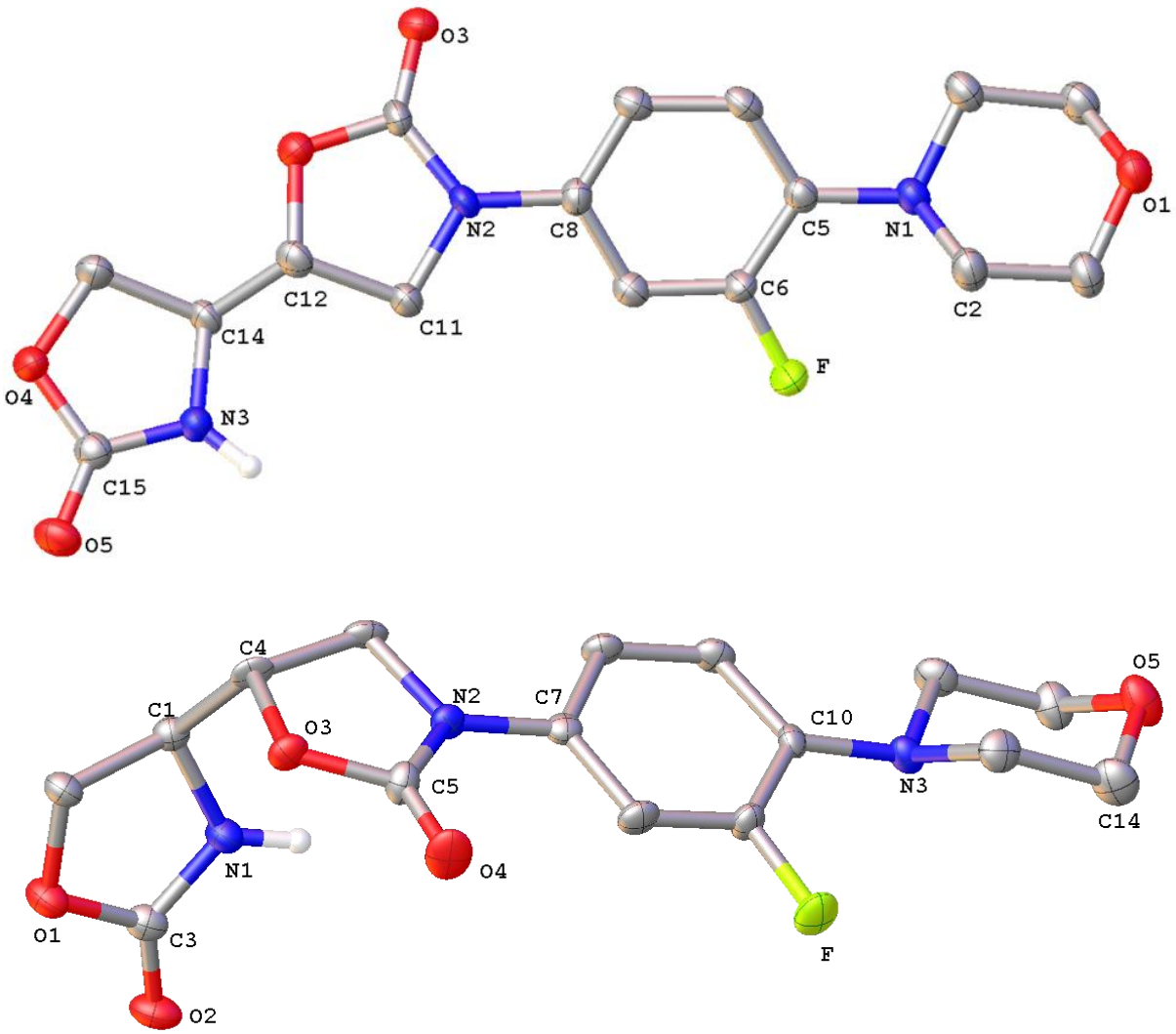
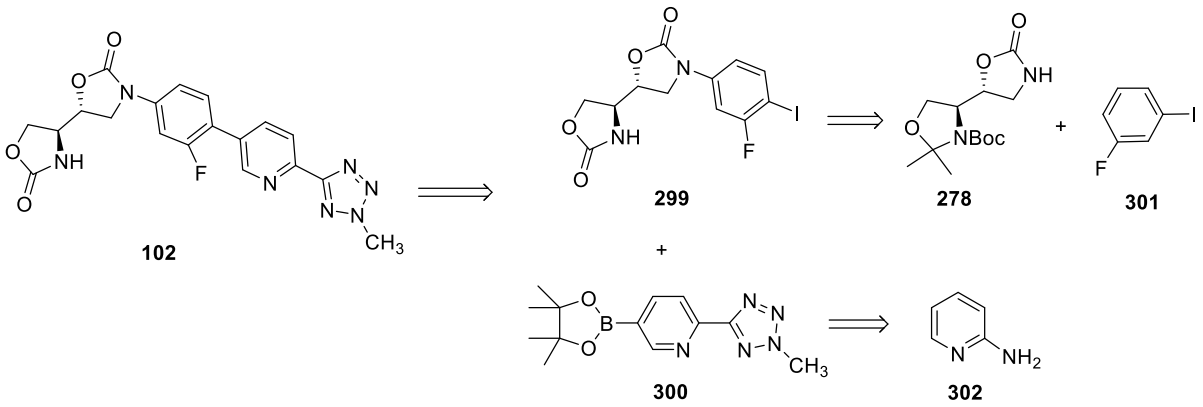


Figure 41 The ORTEP crystal structures of linezolid derivatives **92** (top) and **258** (down). Grey colour stands for carbon, white represents hydrogen atoms, oxygen atoms are painted in red, blue depicts nitrogen and fluoride is coloured in bright green.

2.3.2 Tedizolid derivatives

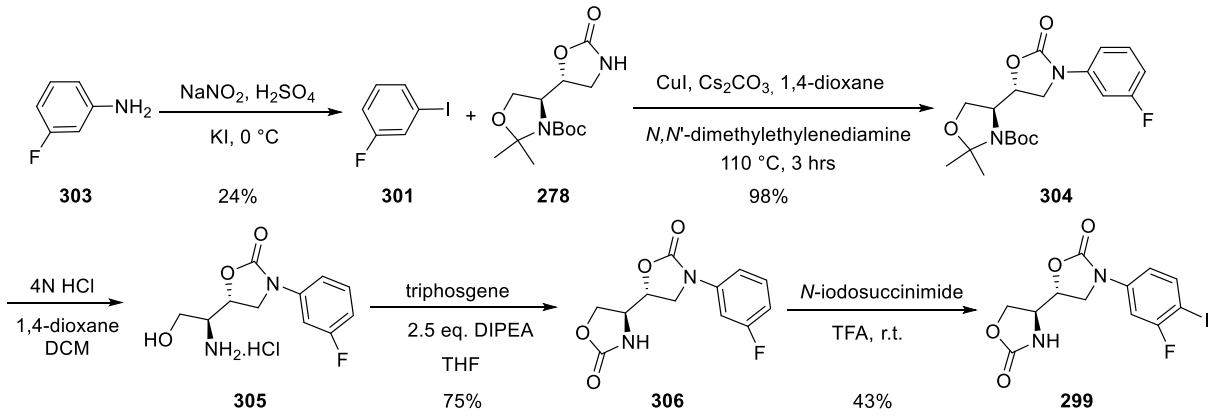
2.3.2.1 Restrosynthetic analysis of tedizolid derivatives



Scheme 31 Restrosynthetic analysis of tedizolid derivative **102**.

A restrosynthesis plan of compound **102** is present in Scheme 31. Compound **102** can be assembled with iodo compound **299** and borane ester **300** via a Suzuki cross coupling reaction. The tetrazolyl pyridine borane ester can be derived from 2-aminopyridine (**303**). On the other hand, a Buchwald-Hartwig amination combines intermediate **278** and 1-fluoro-3-iodobenzene (**301**) to produce **299**.

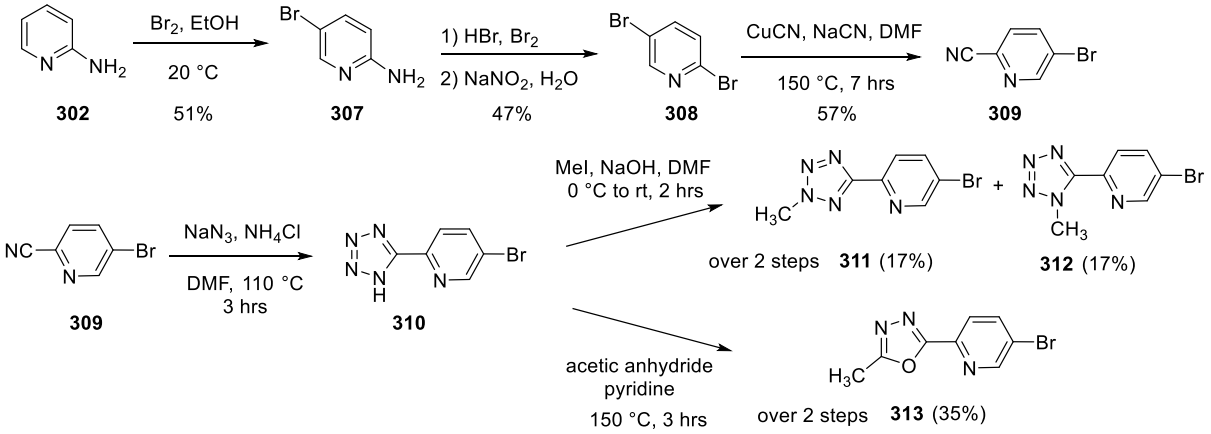
2.3.2.2 Synthesis of iodo compound 299



Scheme 32 Synthesis of iodo compound **299**.

The approach to molecule **299** started from diazotization of 3-fluroaniline (**303**) with sodium nitrite and *para*-toluenesulfonic acid, followed by iodination with potassium iodide leading to 1-fluoro-3-iodobenzene (**301**).[445] Due to its volatility and instability in air, most of the product was vanished via rotary evaporator and only 21% could be obtained. Cross coupling reaction between **301** and **278** under Buchwald conditions resulted in **304** efficiently (98%).[445] Deprotection under strong acidic condition and cyclization with triphosgene and excess *N*,*N*-diisopropylethylamine afforded compound **306** containing two oxazolidinone rings (75% yield over two steps).[461] Then, it was treated with *N*-iodosuccinimide in the presence of trifluoroacetic acid (TFA) to give iodo compound **300** with 43% yield within 3 hours.[462]

2.3.2.3 Synthesis of pyridines 311, 312 and 313

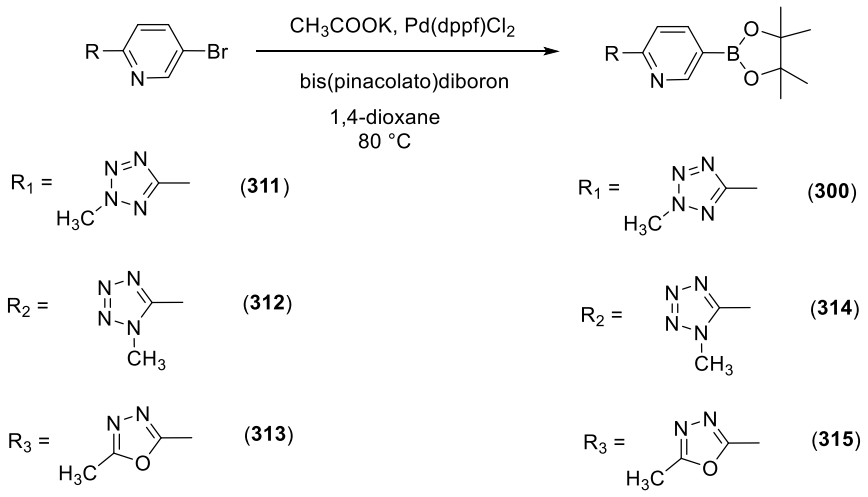


Scheme 33 Synthesis of brominated pyridines **311**, **312** and **313**.

For the pyridine compounds, they were manufactured from commercially available and cheap material, 2-aminopyridine (**302**), via two times brominations, initially *para*-substitution with bromide in cold ethanol to afford 2-amino-5-bromopyridine (**307**) in a moderate yield (51%), followed by substitution of the amino group with bromine atom via diazotization to have 2,5-dibromopyridine (**308**) with 47% yield.[463] Afterwards, the 2-bromo atom was replaced by a cyanide ion using copper cyanide and sodium cyanide as co-reagent under dimethylformamide (DMF) reflux condition for 7 hours[464] and consequently generated 5-bromopicolinonitrile (**309**) in an acceptable yield (57%). The tetrazole ring of 2-(tetrazol-5-yl)-5-bromopyridine **310** was prepared with sodium azide under high temperature reaction condition.[464] Unfortunately, this compound was unstable in column chromatography, thereby crude product was used for next step without further purification. Addition of iodomethane to solution of **310** in cold dimethylformamide produced a mixture of 2-methyltetrazolyl pyridine (**311**) and 1-methyltetrazolyl pyridine (**312**), which was separated by silica-gel column chromatography to yield 17% of each.[464] Oxadiazolyl pyridine **313** was made by rearrangement reaction of **310** with acetic

anhydride under dimethylformamide in reflux condition.[464] The yield over two steps was 35%.

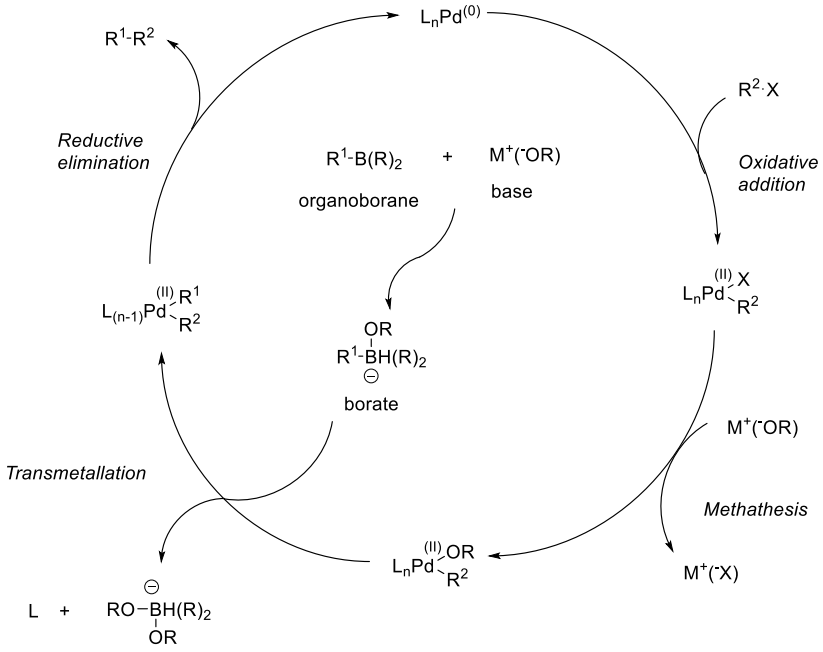
2.3.2.4 Miyaura Borylation



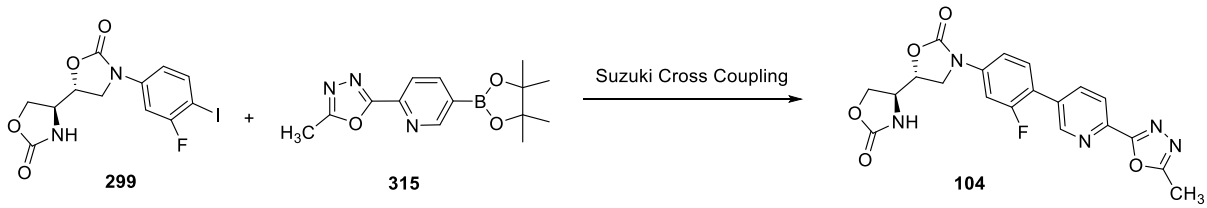
Scheme 34 Borane esterification of **311**, **312** and **313**.

As planned (Scheme 34), organoborane ester **300**, **314** and **315** were obtained by treatment with bis(pinacolato)diboron and potassium acetate under catalysis of Pd(dppf)Cl_2 in warm 1,4-dioxane, respectively.[462] Owing to their instability on silica-gel column chromatography, none of them was purified and directly used in the next step.

2.3.2.5 Synthesis of tedizolid 104 via Suzuki Cross Coupling



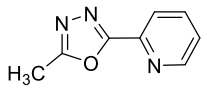
Scheme 35 Mechanism of Suzuki-Miyaura Cross-coupling Reaction.[465]



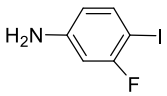
Entry	Catalysts	Reagents	Solvents	Reaction conditions	Yield (%)
1	$Pd(ddf)Cl_2$	K_2CO_3	1,4-dioxane/H_2O (7:1)	80 °C, 16 hrs	0
2	$Pd(ddf)Cl_2$	K_2CO_3	1,4-dioxane/EtOH/H_2O (3:1:1)	40-50 °C, 3 hrs	0
3	$Pd(ddf)Cl_2$	K_2CO_3	1,4-dioxane/EtOH/H_2O (3:1:1)	under reflux, 3 hrs	0
4	$Pd(OAc)_2$	K_2CO_3	DMF/H_2O (1:1)	90 °C, 6 hrs	0
5	$Pd(OAc)_2$/SPhos (1:2)	K_3PO_4	*n*-butanol	100 °C	0
6	$Pd(PPh_3)_4$	Ag_2O	THF/H_2O (10:1)	r.t., 12 hrs	0
7	$Pd(PPh_3)_4$	Na_2CO_3	DMF/H_2O (10:1)	75 °C	0

Table 12 Testing Suzuki reaction conditions for molecule **104**. SPhos: 2-Dicyclohexylphosphino-2′,6′-dimethoxybiphenyl.

The construction of the pivotal C-C bond between the pyridyl and phenyl groups was carried out with a variety of palladium catalyzed Suzuki cross-coupling reaction conditions (Table 12). In first trial, using $Pd(ddf)Cl_2$ as catalyst and potassium carbonate as base, borane ester **315** and aryl iodide **299** were stirred in a degassed solvent mixture of 1,4-dixaone and distilled water in a volume ratio of 7:1 at 80 °C for 16 hours.[466] However, both starting materials were recovered and no reaction was observed. One main reason is the low solubility of the iodo compound. Therefore, ethanol was added into the solvent mixture for improving the solubility and reaction mixture was stirred at around 40-50 °C. GC-MS analysis of reaction residue observed that the borane ester was cleavaged from pyridine ring to reach compound **316**. The reason could be the instability of compound **315** in the GC-MS chamer with high temperatures. Afterwards, harsh condition was applied and the reaction was run under reflux for 3 hours. Apart from **316**, 3-fluoro-4-iodo benzenamine (**317**) was also detected by GC-MS, which was probably derived from completely cleavage of both oxazolidinone rings from molecule **299**.



316



317

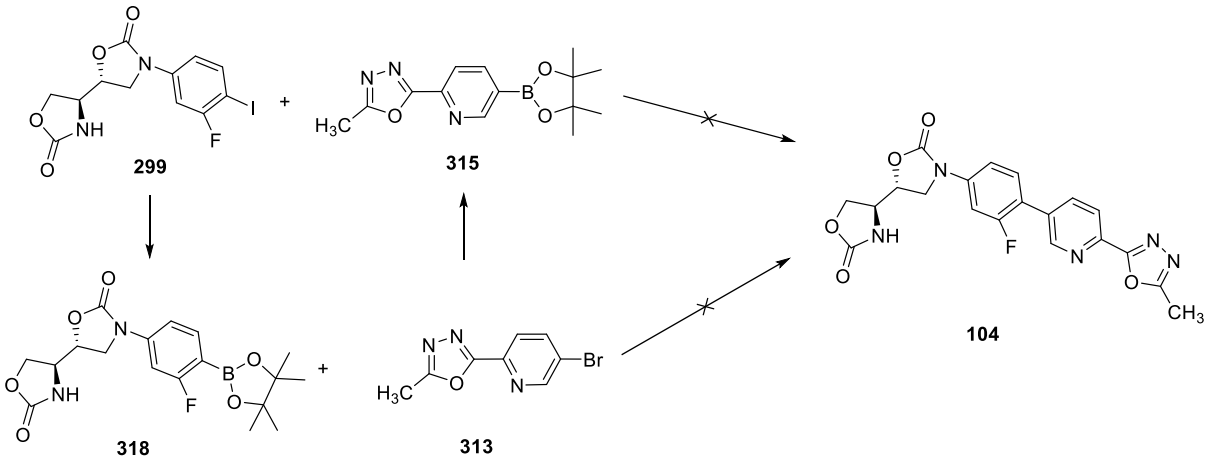
Changing the catalyst into palladium acetate, two differents reaction conditions were tested. One used a solvent mixture of dimethylformamide and distilled water in a volume ratio of 1:1. Indeed, the starting material **299** was better soluble than the previous trials and the reaction was run at 90 °C for 6 hours. However, in this case, no product was achieved either.[462] On the other hand, a combination of palladium and diakylbiphenylphosphino ligands (SPhos) was reported

with highly catalytic activity to proceed these coupling reactions.[467] It was also performed in practical trial but failed.

In addition, two cases with tetrakis(triphenylphosphine)palladium(0) were introduced into the tests. One was treated with $Pd(PPh_3)_4$ and silver(I) oxide in solvent mixture of tetrahydrofuran and distilled water (10:1) and stirred at room temperature for 20 hours.[468] Compound **316** was identified from the reaction residue by GC-MS and no more products could be isolated. In another case, $Pd(PPh_3)_4$ and sodium carbonate were employed and reaction mixture was stirred in dimethylformamid and distilled water (10:1) at 70 °C overnight.[469] Still, no reaction took place.

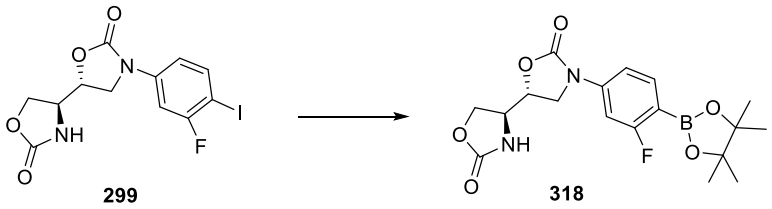
The reasons for the failure of the Suzuki cross coupling could be various. For instance, the insolubility of the iodo compound **299** in most common organic solvents, in which the two oxazolidinone rings might deactive the molecule reactivity, thereby the reaction couldnot be proceeded as expected. The incompletely degassed solvents could be another impact on this, because Suzuki coupling reaction has to be done in an extremely anaerobic environment.

2.3.2.6 Alternative solution to oxazolidinone 104



Scheme 36 Alternative solution to afford oxazolidinone **104**.

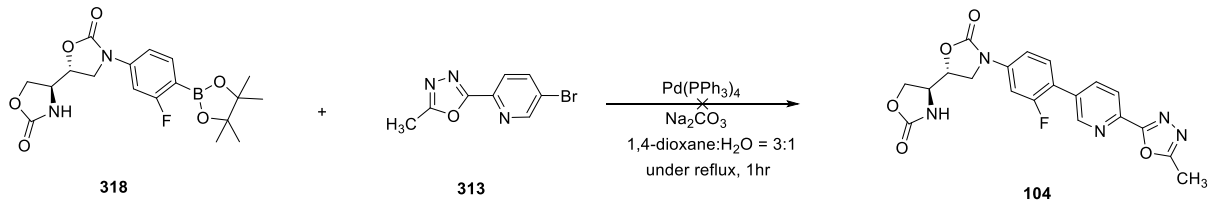
Since the Suzuki coupling reaction between **299** and **315** did not give a satisfied result, the alternative solution was concerned that iodo compound **299** was initially borylated into **318** and further performed the Suzuki cross coupling reaction with **313** (Scheme 36). Several conditions were conducted (Table 13).



Entry	Catalysts	Reagents	Solvents	Reaction conditions	Yield (%)
1	CuI	NaH, pinacolborane	THF	r.t.	0
2	$Pd(ddf)Cl_2$	TEA, bis(pinacolato)diboron	1,4-dioxane	under reflux, 7 hrs	0
3	$Pd(ddf)Cl_2$	CH_3COOK, bis(pinacolato)diboron	1,4-dioxane	80 °C, 3 hrs	0
4	$Pd(ddf)Cl_2$	CH_3COOK, bis(pinacolato)diboron	DMSO	80 °C, 3 hrs	75

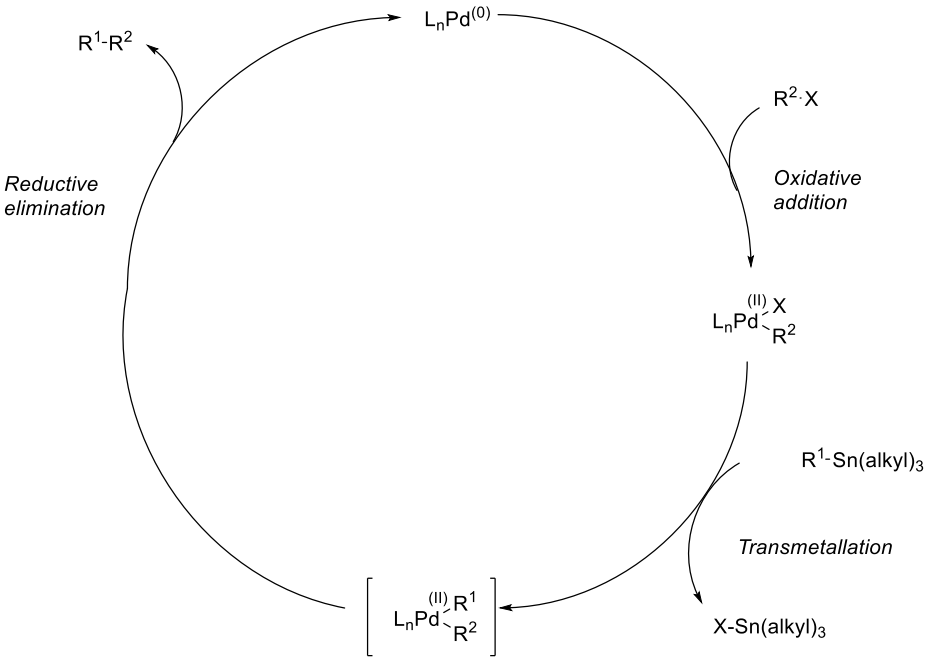
Table 13 Formation of boronate ester **318** via Miyuara borylation.

In the first entry, formation of arylboronate **318** was made by a CuI-catalyzed coupling reaction of pinacolborane under the protonation of sodium hydride at room temperature.[470] After stirring several days, around 74% of the starting material was recovered and no desired product was provided. Two more common borylation reactions with bis(pinacolato)diboron, $Pd(ddf)Cl_2$ and different bases (triethylamine or potassium acetate)[471] were performed. The prior was heated under reflux for 7 hours and the latter one was run at 80 °C for 3 hour. However, no product was found. It was noticed that in each case, iodo compound **299** exhibited extremely poor solubility in common organic solvents, for example, tetrahydrofuran, 1,4-dioxane and dichloromethane, further influenced the molecule reactivity. Thus, dimethylsulfoxid was utilized in the reaction.[472] NMR analysis indicated that **318** was generated and contained 75% proportion of the entire reaction residue. Due to the possible instability in column chromatography on silica gel, boronate ester **318** was used for Suzuki cross coupling reaction without purification. It was stirred with brominated pyridine **313** by treatment with $Pd(PPh_3)_4$ as catalyst and sodium carbonate as base in the degassed solvent mixture of 1,4-dioxane and distilled water (3:1) and heated for 1 hour.[473] It existed only compound **317** after purification by column chromatography.



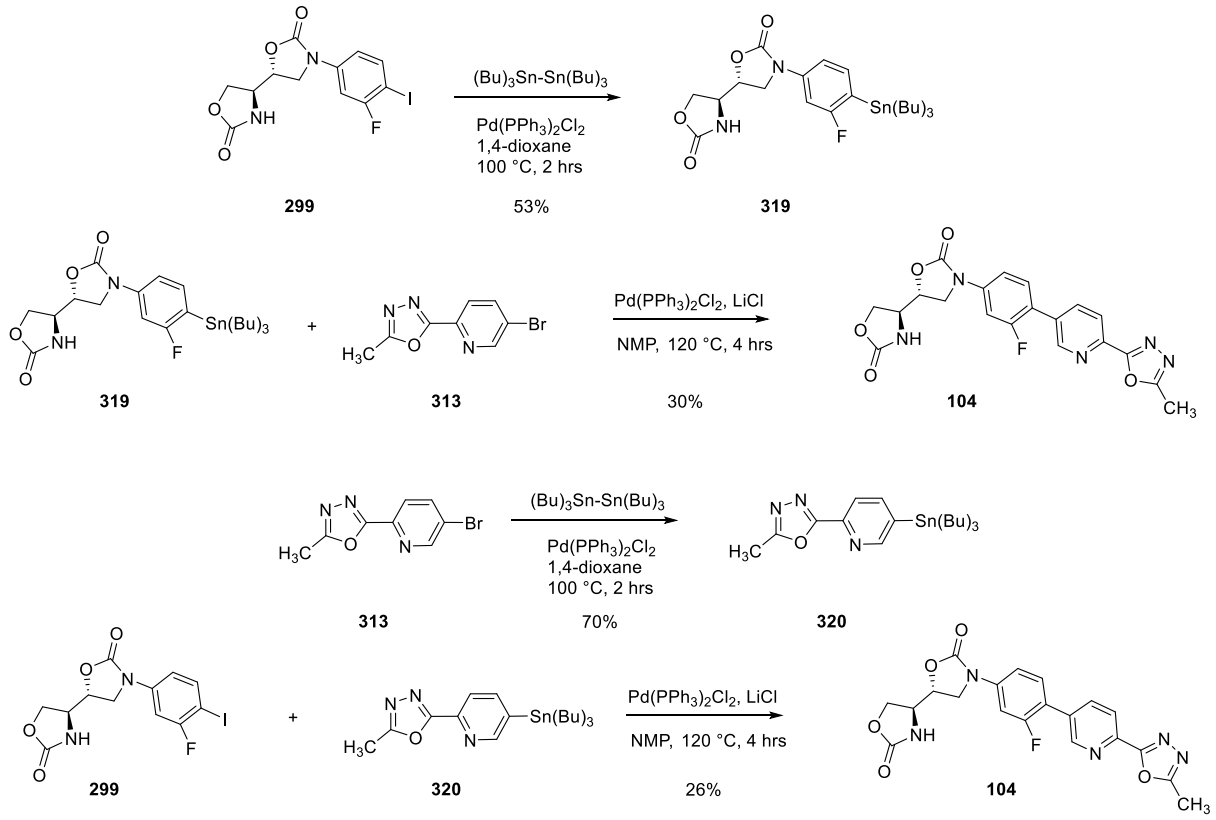
Scheme 37 Suzuki cross coupling between **318** and **313**.

2.3.2.7 Synthesis of tedizolid derivatives via Stille reaction



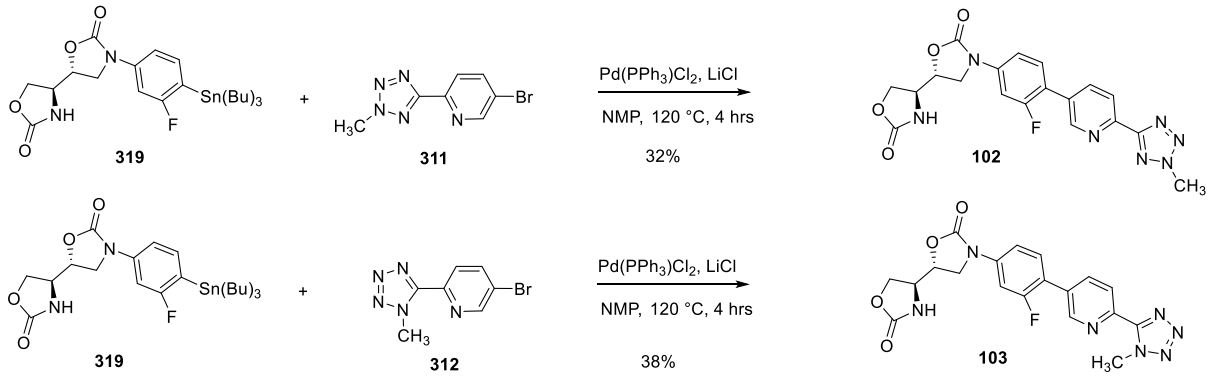
Scheme 38 Mechanism of Stille cross coupling reaction.[465]

Rhee and Im disclosed the first route to gain tedizolid by employment of Stille cross coupling reaction.[465],[474] However, a poor yield and the residual tin impurity contained in the active pharmaceutical ingredient (API) drove the research into a facile, economical and environmentally benign approach.[464],[474] Therefore, Suzuki coupling was introduced by Huang as an alternative improved methodology.[462] Since it did not accomplish the expectation in the constitution of target compounds **102**-**104** and has become problematic to some extent, Stille coupling was reconsidered into the entry.



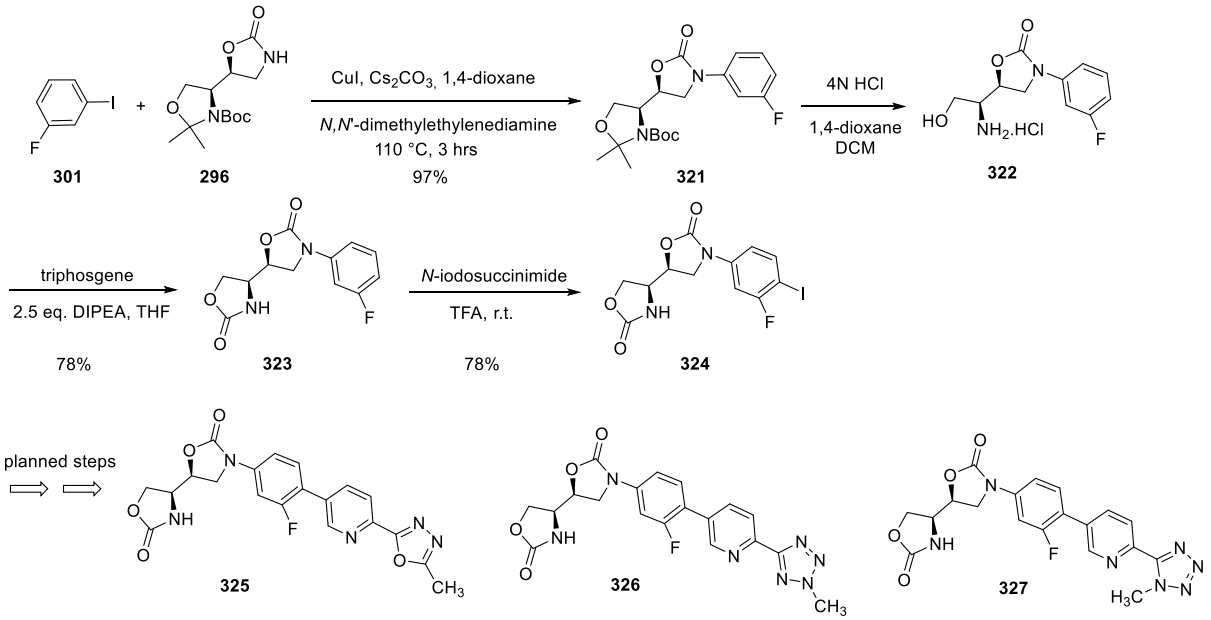
Scheme 39 Formation of tedizolid derivative **104** via Stille coupling.

As illustrated in Scheme 39, two possible routes can afford **104**. Iodo compound **299** was converted into organostannane **319** in an acceptable yield (53%) by treatment with hexabutylditin and bis(triphenylphosphine)palladium (II) dichloride as catalyst in hot 1,4-dioxane for 2 hours. After that, Stille coupling of organostannane **319** and brominated pyridine **313** with bis(triphenylphosphine)palladium (II) dichloride and lithium chloride in hot *N*-methy-2-pyrrolidone for 4 hours resulted final product **104** with a poor yield (30%). It also can attach tributyltin group firstly to **313**, in return organotin **320** was afforded in a better yield (77%), further coupled with iodo compound **299** via a Stille reaction to reach **104** in a low yield (26%).



Scheme 40 Formation of tedizolid derivatives **102** and **103** via Stille coupling.

The similar strategy can be implemented to generate the other two tedizolid derivatives. 5-Bromo-2-(2-methyl-2*H*-tetrazol-5-yl)pyridine (**311**) and 5-bromo-2-(1-methyl-1*H*-tetrazol-5-yl)pyridine (**312**) were treated with organostannane **320** under the Stille reaction condition, respectively. After 4 hours reaction time, compounds **102** and **103** were all yielded (32% and 38%, separately). The results of Stille coupling were still imperfect, including low conversion with poor yield, complicated and toxic operation, and difficult purification containing tin residues. Therefore, either the reaction condition is necessary to be optimized or other cross coupling reaction for construction of carbon-carbon bond should be concerned, such as Negishi coupling between an organohalide and an organozinc compound, and Hiyama coupling between organohalides and organosilicon compound, thereby the coupling fragments have to be modified accordingly.



Scheme 41 Formation of isomers **325**, **326**, and **327**.

Isomers of tedizolid derivatives can also be obtained via this synthetic pathway (in Scheme 41). Buchwald cross coupling of fragments **301** and **296** gave the compound **321** in an excellent yield (97%),[445] followed by deprotection under acidic condition and cyclization with triphosgene to afford oxazolidinone **322** in a moderate yield (78% over two steps).[461] Iodination of **323** with *N*-iodosuccinimide in the presence of trifluoroacetic acid (TFA) at room temperature converted into corresponding iodo compound **324** efficiently (78%).[462] Afterwards, the future synthetic plan is to transform **324** into tin substitutes, followed by Stille coupling reaction with brominated pyridines **311**-**313** can deliver substances **325**, **326** and **327**.

2.4 Bioactivity Test

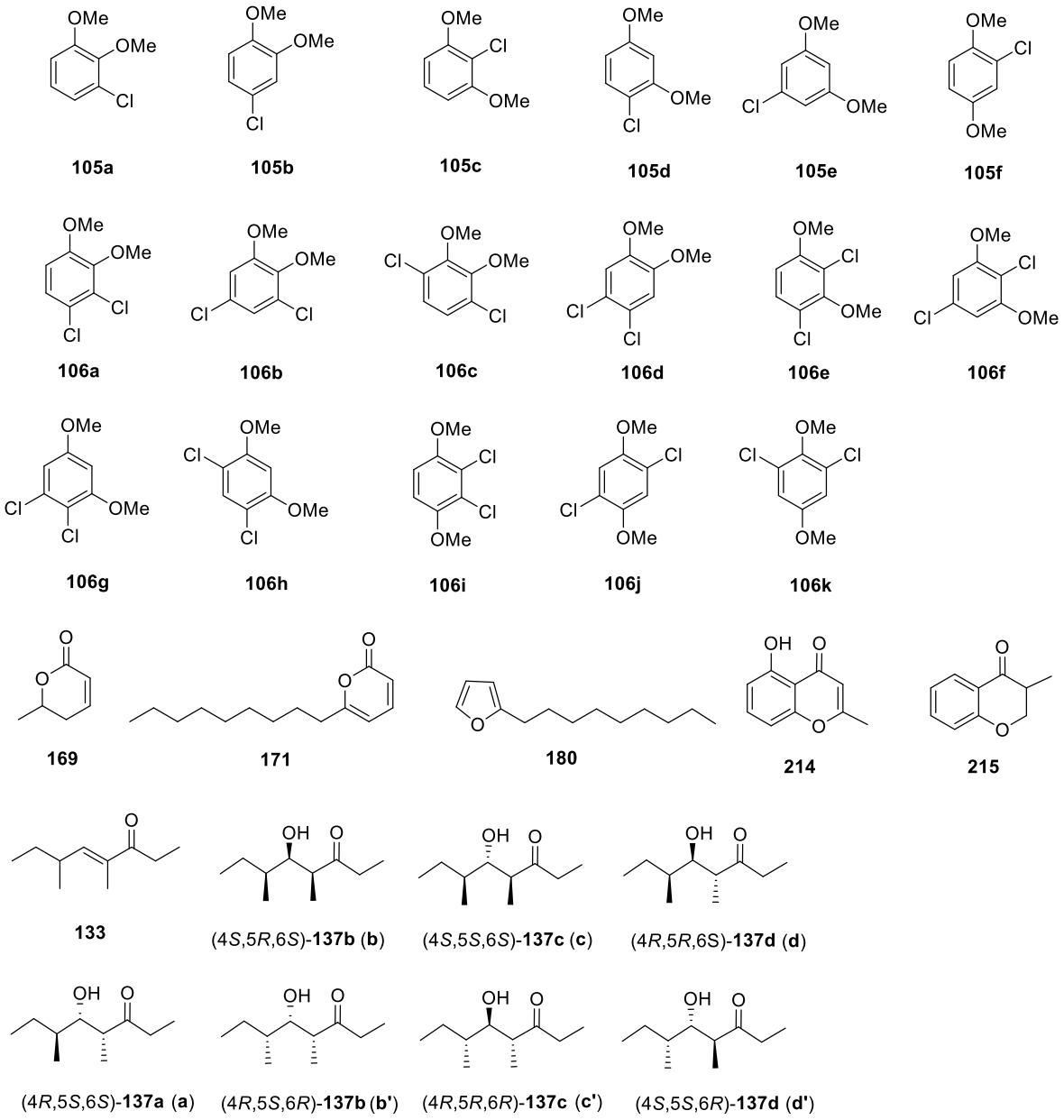


Figure 42 The fungal metabolites and their related compounds, of which biological antibacterial and antifungal activity tests in *vitro* were performed.

The determination of minimum inhibitory concentration (MIC) value is the priority biological data in a program aiming at discovering novel antibacterial agents. The *in vitro* antibacterial and antifungal activity of 43 authentic synthetic samples and natural products obtained from three different projects, comprised of 17 chlorinated aromatic compounds, 2 lactones, 1 pyran, 10 oxazolidinones, 3 morpholin aromatic substances,

2 chromanes, and 8 ketones (seen in Figures 42 and 43), against a panel of common fungal and bacterial microorganisms was evaluated and presented in Tables 14-17. The involved fungal strains embrace *Schizosaccharomyces pombe*, *Pichia anomal*, *Mucor hiemalis*, *Candida albicans*, and *Rhodotorula glutinis*. Gram-positive strains tested included *Micrococcus luteus*, *Bacillus subtillis*, *Staphylococcus aureus*, *S. aureus MRSA*, *Mycobacterium sp.*, and *Mycobacterium smegmatis*, while the selected Gram-negative organisms were limited to *Escherichia coli*, *Chromobacterium violaceum* and *Pseudomonas aeruginosa*. Five control drugs were used as references, such as Oxytetracyclin hydrochlorid, Gentamycin, Nystatin, Kanamycin, and Linezolid.

The result indicated that among the chlorinated aromatic compounds, several species exhibited potency in particular against fungal organisms (Tables 14 and 15). For instance, the MIC value of 2,5-dichloro-1,3-dimethoxybenzene (**106f**), 1,5-dichloro-2,4-dimethoybenzene (**106h**) and 1,4.dichloro-2,5-dimethoybenzene (**106j**) for fungal strain *Pichia anomala* was at 33.3 μg/mL, which shared the same value as the antifungal medication, Nystatin. 2-Chloro-1,3-dimethoxybenzene (**105c**) and 1,2-dichloro-3,5-dimethoxybenzene (**106g**) however against the same fungal species with less activity than the formers. For *Mucor hiemalis*, majority of chlorinated molecules displayed inhibition activity, such as 1,3-dichloro-2,4-dimethoxybenzene (**106e**), 1,2-dichloro-3,5-dimethoxybenzene (**106g**) and 1,3-dichloro-2,5-dimethoybenzene (**106k**) owned the MIC value at 33.3 μg/mL, while 2-chloro-1,3-dimethoxybenzene (**105c**), 1,2-dichloro-3,4-dimethoxybenzene (**106a**), 1,2-dichloro-4,5-dimethoxybene (**106d**) and 2,5-dichloro-1,3-dimethoxybenzene (**106f**) only reached 67.0 μg/mL. Nevertheless, all of the values were still higher than control pills Nystatin in the aspect of antifungal activity. Besides, 1,2-dichloro-3,4-dimethoxybenzene (**106a**)

and 1,3-dichloro-2,5-dimethoybenzene (**106k**) exhibited antifungal character against *Candida albicans*, whilst they also showed antibacterial function to Gram-negative bacteria *Chromobacterium violaceum*. Additionally, **106e** behaved weak antibacterial activity against *Chromobacterium violaceum*. But these numbers were not so attractive for emerging a new generation of remedies either. Unfortunately, the identified natural products, 4-chloro-1,2-dimethoxybenzene (**105b**) and 1,5-dichloro-2,3-dimethoxybenzene (**106b**) emitted by the fungus *Geniculosporium* did not restrain the growth of any tested strains and no inhibition was observed.

The natural products released and identified from Xylariaceae family brought out interesting achievements as well (Tables 15 and 17). Two lactones **169** and **171** were examined and delivered with total different outcome. It contained no activity against the selected microorganisms in the former lactone. In contrast, 6-nonyl-2*H*-pyran-2-one (**171**) is more active in this field. It exhibited potent antifungal activity against fungal species *Schizosaccharomyces prombe*, *Mucor hiemalis*, *Candida albicans* and *Rohodotorula glutinis*, whose value is equal or even much better than reference compound Nystain, displayed moderate antifungal activity against *Pichia anomala* and weak antibacterial property against Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*. 2-Nonylfuran (**180**) has moderate antifungal activity against *Mucor hiemalis*. Afterwards, the moderate antifungal activity belongs to two chromanes **214** and **215** against fungal organisms *Mucor hiemalis* and *Rhodotorula glutinis*. It did not detect any antifungal or antibacterial activity of Ant alarm pheromones, manicone (**133**) against the chosen microorganisms. This also happened in natural product **137** ((4*R*,5*R*,6*S*)-**137d**) as well as their synthesized isomers.

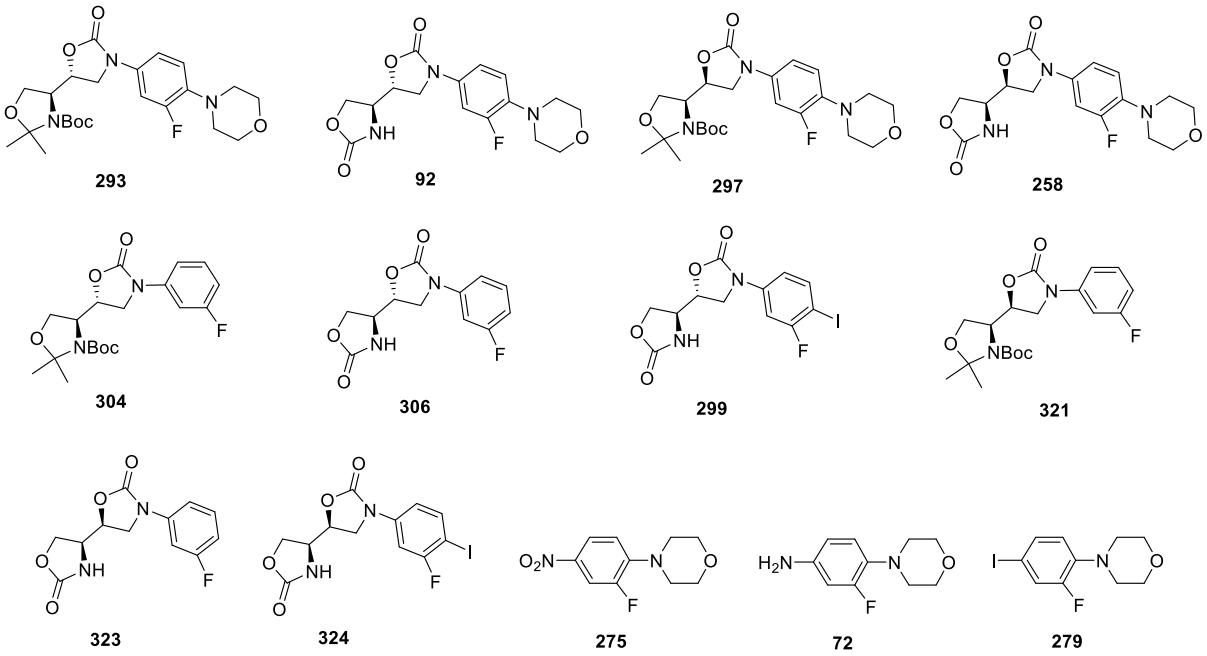


Figure 43 The synthetic oxazolidinone substances and intermediates envolved in the production were evaluated for antimicrobial and cytotoxic activities.

In addition, a series of the synthetic oxazolidinone analogs and intermediates with potential antibiotic ability were tested for their antifungal and antibacterial activity (Tables 16 and 17). To be surprised and frustrated, the linezolid derivative **92** with the most promising theoretical calculation result yet failed the activity tests and no inhibition towards the selected fungal strains, Gram-positive and Gram-negative pathogens was discovered. The same problem occurred in its precursor **293**. On the contrary, their isomers **297** and **258** revealed weak antifungal activity towards *Mucor hiemalis* and *Rhodotorula glutinis*. These results were completely beyond the expectation. In the following screening tests, compound **304** was found out to own the weak antifungal activity against *Mucor hiemalis*. Dioxazolidinone ring substance **306** showed no inhibition in searching the biological activity. Its iodo substitutes **299**, however, displayed moderate antifungal activity towards all chosen fungal microorganisms, among which had strongest effected on the growth of *Mucor hiemali*, mildly against *Candida albicans*,

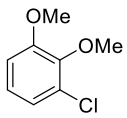
and gently treated *Schizosaccharomyces prombe*, *Pichia anomala* and *Rhodotorula glutinis*. It also displayed antibacterial activity against Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *S. aureus MRSA*, *Micrococcus luteus* and Gram-negative bacteria *Chromobacterium violaceum*. The oxazolidinone analogues with different stereochemical center, such as **321**, **323** and **324**, however were not able to develop any bioactivity against the screened microorganisms.

Apart from that, the morpholine aromatic compounds were also assessed. Only 4-(2-fluoro-4-nitrophenyl)morpholine (**275**) and 4-(2-fluoro-4-iodophenyl)morpholine (**279**) exhibited weak antifungal activity towards *Mucor hiemalis* and *Rhodotorula glutinis*. No inhibition was investigated from 3-fluoro-4-morpholinoaniline (**72**).

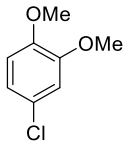
To sum up briefly, a total of 43 chemicals, either volatile natural products or authentic synthetic substances obtained from 3 different projects were screened in evaluating their activity in effecting the growth of 14 distinct organisms, including 5 fungal strains, 6 Gram-positive bacteria and 3 Gram-negative bacteria. In general, the chlorinated aromatic compounds perform better antifungal ability and three of them (**106f**, **106h** and **106j**) own the same minimum inhibitory concentration by treatment with *Pichia anomala* as the control drugs, Nystatin. In contrast, the identified natural products (**105b** and **106b**) from the fungus *Geniculosporium* did not show any inhibitory effects. The volatiles from headspace analysis of fungal Xylariaceae family behave mostly against fungal expansion, especially 6-nonyl-2*H*-pyran-2-one (**171**) manifested antifungal activity towards certain fungal species. It was more disappointing that except chemical intermediate **299** with moderate property in those biotests, nearly none of oxazolidinone derivatives obtained via synthesis delivered interesting results.

Test organism (μg /mL)	DSM	105a	105b	105c	105d	105e	105f	106a	106b	106c	106d	Reference
Schizosaccharomyces prombe	70572	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	42.0 – 67.0 [c]
Pichia anomala	70572	n.i.	n.i.	**67.0**	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	33.3 [c]
Mucor hiemalis	2656	n.i.	n.i.	**67.0**	n.i.	n.i.	n.i.	**67.0**	n.i.	n.i.	**67.0**	5.26 – 16.6 [c]
Candida albicans	1665	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	**67.0**	n.i.	n.i.	n.i.	33.3 [c]
Rhodotorula glutinis	10134	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	≤ 0.52 – 16.7 [c]
Micrococcus luteus	20030	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	0.42 [a]
Bacillus subtilis	10	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	6.7 – 8.3 [a]
Escherichia coli	1116	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	0.83 [a]
Staphylococcus aureus	346	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	0.1 – 0.21 [a]
Mycobacterium sp.	43270	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	2.1 [a]
Chromobacterium violaceum	30191	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	**33.3**	n.i.	n.i.	n.i.	0.83 – 8.3 [a]
Pseudomonas aeruginosa	50071	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	16.6 – 21.0 [b]
cell line L929 IC50		>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	

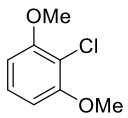
Table 14 *In vitro* antibacterial and antifungal activity. n.i.: no inhibition. MIC: Minimum inhibitory concentration; [a]Oxytetracyclin hydrochloride; [b]Gentamycin; [c]Nystatin; DMSZ: German Collection of Microogranisms and Cell Cultures Braunchweig.



105a



105b



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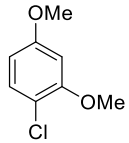

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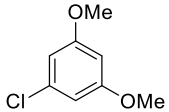

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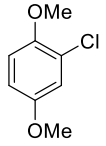
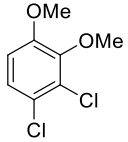


105f



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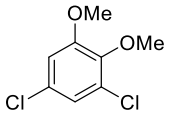
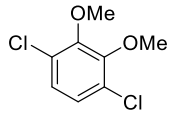
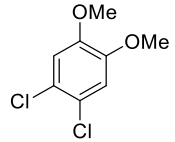


106b



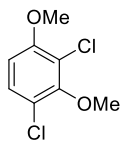
106c



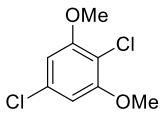
106d

Test organism (μg /mL)	DSM	106e	106f	106g	106h	106i	106j	106k	169	171	180	Reference
Schizosaccharomyces prombe	70572	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	**67.0**	n.i.	**16.6**	n.i.	42.0 – 67.0 [c]
Pichia anomala	70572	n.i.	**33.3**	**67.0**	**33.3**	n.i.	**33.3**	n.i.	n.i.	**33.3**	n.i.	33.3 [c]
Mucor hiemalis	2656	**33.3**	**67.0**	**33.3**	n.i.	n.i.	n.i.	**33.3**	n.i.	**16.6**	**16.6**	5.26 – 16.6 [c]
Candida albicans	1665	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	**67.0**	n.i.	**16.6**	n.i.	33.3 [c]
Rhodotorula glutinis	10134	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	**16.6**	n.i.	≤ 0.52 – 16.7 [c]
Micrococcus luteus	20030	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	0.42 [a]
Bacillus subtilis	10	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	**67.0**	n.i.	6.7 – 8.3 [a]
Escherichia coli	1116	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	0.83 [a]
Staphylococcus aureus	346	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	**67.0**	n.i.	0.1 – 0.21 [a]
Mycobacterium sp.	43270	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	2.1 [a]
Chromobacterium violaceum	30191	**67.0**	n.i.	n.i.	n.i.	n.i.	n.i.	**67.0**	n.i.	n.i.	n.i.	0.83 – 8.3 [a]
Pseudomonas aeruginosa	50071	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	16.6 – 21.0 [b]
cell line L929 IC50 1000 μg/ml		>10	>10	>10	>10	>10	>10	>10	**6**	>10	>10	
cell line L929 IC50 10000 μg/ml*									**3.1**	30		

Table 15 *In vitro* antibacterial and antifungal activity. n.i.: no inhibition. MIC: Minimum inhibitory concentration; [a]Oxytetracyclin hydrochloride; [b]Gentamycin; [c]Nystatin; DMSZ: German Collection of Microogranisms and Cell Cultures Braunchweig ; * this test was only repeated with **169** and **171**.



106e



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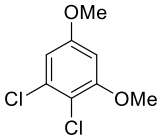

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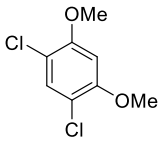

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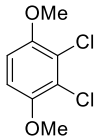
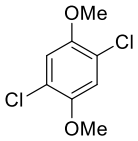
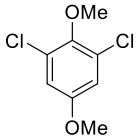


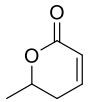
106i



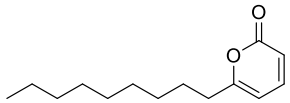
106j



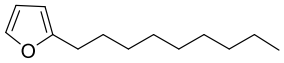
106k



169



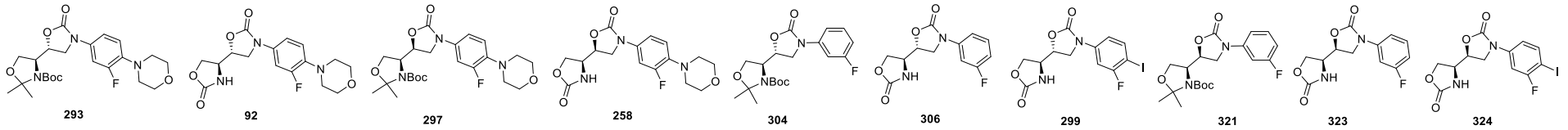
171



180

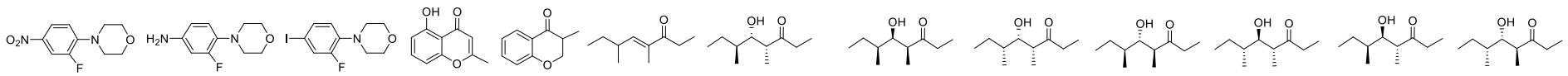
Test organism (μg /mL)	DSM	293	92	297	258	304	306	299	321	323	324	Refenrence
Staphylococcus aureus	346	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	**33.3**	n.i.	n.i.	n.i.	0.21; 2.1 [a],[e]
S. aureus MRSA	11822	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	**67.0**	n.i.	n.i.	n.i.	4.2; 2.1 [f],[e]
Mycobacterium smegmatis	ATCC 70084	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	8.3; 2.1 [d],[e]
Bacillus subtilis	10	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	**33.3**	n.i.	n.i.	n.i.	4.2; 0.42 [a],[e]
Micrococcus luteus	1790	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	**67.0**	n.i.	n.i.	n.i.	0.83; 2.1 [a],[e]
Escherichia coli	1116	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	1.7; / [a],[e]
Chromobacterium violaceum	30191	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	**67.0**	n.i.	n.i.	n.i.	0.42; 67.0 [a],[e]
Pseudomonas aeruginosa	PA14	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	0.42; / [b],[e]
Schizosaccharomyces pombe	70572	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	**67.0**	n.i.	n.i.	n.i.	16.6; / [c],[e]
Pichia anomala	6766	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	**67.0**	n.i.	n.i.	n.i.	8.3; / [c],[e]
Mucor hiemalis	2656	n.i.	n.i.	**67.0**	**67.0**	**67.0**	n.i.	**16.6**	n.i.	n.i.	n.i.	8.3; / [c],[e]
Candida ablbicans	1665	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	**33.3**	n.i.	n.i.	n.i.	16.6; / [c],[e]
Rhodotorula glutinis	10134	n.i.	n.i.	**67.0**	n.i.	n.i.	n.i.	**67.0**	n.i.	n.i.	n.i.	16.6; / [c],[e]

Table 16 *In vitro* antibacterial and antifungal activity. n.i.: no inhibition. MIC: Minimum inhibitory concentration; [a]Oxytetracyclin hydrochloride; [b]Gentamycin; [c]Nystatin; [d]Kanamycin; [e]Linezolid. DMSZ: German Collection of Microogranisms and Cell Cultures Braunchweig.



Test organism (μg /mL)	DSM	275	72	279	214	215	133	a	b	b'	c	c'	d	d'	Acetone 20 μl	Acetone/10% DMSO 20 μl
Staphylococcus aureus	346	n.i.	n.i.	n.i.	**67.0**	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
S. aureus MRSA	11822	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Mycobacterium smegmatis	ATCC 70084	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Bacillus subtilis	10	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Micrococcus luteus	1790	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Escherichia coli	1116	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Chromobacterium violaceum	30191	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Pseudomonas aeruginosa	PA14	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Schizosaccharomyces pombe	70572	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Pichia anomala	6766	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Mucor hiemalis	2656	**67.0**	n.i.	**67.0**	**33.3**	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Candida ablbicans	1665	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Rhodotorula glutinis	10134	**67.0**	n.i.	**67.0**	**33.3**	**67.0**	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.

Table 17 *In vitro* antibacterial and antifungal activity. n.i.: no inhibition. MIC: Minimum inhibitory concentration; [a]Oxytetracyclin hydrochloride; [b]Gentamycin; [c]Nystatin; [d]Kanamycin; [e]Linezolid. DMSZ: German Collection of Microogranisms and Cell Cultures Braunchweig.



3. Conclusion

In this thesis, three different projects were conducted individually. First project described the elucidation of the structures of two suspected volatile substances released by the endophytic fungus *Geniculosporium* sp. 9910. They were chlorinated dimethoxybenzenes according to the suggestions of the mass spectral libraries. Therefore, the constitutional isomers of both volatiles (Figure 19) were obtained via synthesis (Scheme 8 and 9) or acquired from commercial suppliers. The unambiguous structures of the natural products **105** and **106** were clarified as 4-chloro-1,2-dimethoxybenzene (**105b**) and 1,5-dichloro-2,3-dimethoxybenzene (**106b**) by the judgement of their mass spectra and retention indices.

In the second case, a panel of volatile organic compounds were emitted from 14 xylariaceous fungal microorganisms and analyzed by GC-MS. A collection of 88 substances from multiple categories was ultimately determined, including fatty acid derivatives (alcohols, aldehydes and ketones, esters), lactones, furans, nitrogen and sulfur compounds, aromatic compounds and terpenoids. For certain species, synthetic approaches and purchased chemicals were utilized to prove the predictions by mass spectral libraries. For instance, manicone **133** was reached by five-step synthesis (Scheme 10) from commercial available 2-methylbutanal (**140**). However, the process of identification of two alcoholic ketone **137** was severely complicated and bumpy. Initially, all eight stereoisomers of **137** were achieved from an aldol addition and separately by GC on a homochiral stationary phase. One of them was considered as natural product accordling to its GC retention time. Later, two isomers ((4*R*,5*S*,6*S*)-**137a** and (4*S*,5*R*,6*S*)-**137b**) were afforded

through stereoselective synthesis (Scheme 12), yet the result of chiral GC excluded the possibility due to different retention time compared to natural products. The task was subsequently accomplished by extensive chromatographic purification of racemic mixture of eight stereoisomers. Afterwards, (4*R*,5*R*,6*S*)-5-hydroxy-4,6-dimethyloctan-3-one ((4*R*,5*R*,6*S*)-**137d**) was matched to natural product **137** based on the retention time in chiral GC spectra (Figure 26). The structures of other organic volatile compounds were elucidated much more easily. 6-Methyl-5,6-dihydro-*2H*-pyran-2-one (**169**), 6-nonyl-2*H*-pyran-2-one (**171**) and 2-nonylfuran (**180**) were produced in either one- or two-step synthesis (Scheme 15 and 16) and determined by their mass spectra and retention indices. For aromatic compounds, structurally related compounds **194**, **197**, **198**, **203** and **205** were identified as volatile organic compounds by commercial available authentic samples while 5-hydroxy-2-methyl-4*H*-chromen-4-one (**214**) and 2-methyl-4-chromanone (**215**) were generated by one-step synthesis (Scheme 18 and 19) and also showed up in the headspace analysis.

In the last programm, the synthesis of linezolid derivative **92** predicted in silico was performed. The key steps were the introduction of the oxazolidinone ring and the formation of C-N bonding. First entry was to have the 2-oxazolidone initially, further epoxidation followed by nucleophilic attack from 3-fluoro-4-morpholinoaniline (**72**). A variety of reaction conditions were performed but yet resulted in no desired product. The route was therefore modified to yield epoxides **280** and **281** with the oxazolidine ring at the first place, subsequently attacked by aqueous ammonia to reach the amino alcohol **282**, then cyclization into the first oxazolidinone ring **278**. Buchwald cross coupling under the catalysis of copper iodide joined the two fragments **278** and **279**. Strong acid opened the oxazolidine ring and built the second oxazolidinone ring

by usage of triphosgene in excess Hünig's base. In the consequence, compound **92** was generated in 13 steps as the longest linear sequence. Similar synthetic method was applied in the production of its isomer **258**. The crystal structures of both molecules were obtained and determined to confirm the stereochemical issues.

The construction of tedizolid derivatives **102**, **103** and **104** was primarily planned to combine iodo compound **299** and pyridine borante ester **300** via Suzuki-Miyaura cross coupling reaction. Substance **304** was reached by Buchwald cross coupling between 1-fluoro-3-iodobenzene (**301**) and oxazolidinone **278**, followed by deprotection, cyclization and iodination. In the other hand, commercially available and cheap chemical, 2-aminopyridine (**302**) was processed via bromination, cyanation, and later was treated with sodium azide to afford the tetrazole ring compound **310**. It was treated with either iodomethane or acetic anhydride to yield 2-methyltetrazolyl pyridine (**311**) and 1-methyltetrazolyl pyridine (**312**) or oxadiazolyl pyridine **313**, respectively. Afterwards, Miyaura borylation of those pyridines yielded the corresponding organoborane ester **300**, **314** and **315**. The next step with palladium catalyzed Suzuki-Miyaura cross-coupling reaction failed. The reason could be the insolubility iodo compound **299** owing to the two oxazolidinone rings and the incompletely degassed solvents. The borylation of **299** was also made only in DMSO solvent and the conversion was around 75%. Unfortunately, the following Suzuki reaction didnot take place as expected. Intentionally, Stille coupling was executed instead, including transformation of iodo compound **299** or brominated pyridine **313** into corresponding organostannane **318** or **315**, followed by C-C bond coupling with bis(triphenylphosphine)palladium (II) dichloride and lithium chloride to afford target molecule **104** in poor yield. This was also utilized in terms of other tedizolid derivatives **102** and **103**. The purification of the

products was not performed very successfully. Therefore, none of them was pure and clean enough in pursuit of their specific bioactivity in biotests. The optimization of the Stille reaction condition, improvement of purification methods for the products or even other C-C bond coupling attempts are demanded to complete the route.

Afterwards, the bioassays of the volatile organic compounds from fungi and the synthetic products or intermediates involved into the total synthesis of oxazolidinone derivatives were made. Mainly, the antifungal and antibacterial activity of these compounds against selected common fungal organisms, Gram-positive and Gram-negative pathogens was evaluated. The reports demonstrated that 6-nonyl-2*H*-pyran-2-one (**171**) identified from xylariaceous fungi behaved the moderate bioactivity against most fungal microorganisms and weak antibacterial effect towards Gram-positive bacteria. The lactone **169** was moderately cytotoxic, but devoid of significant antimicrobial activity. The chlorinated dimethoxybenzenes (**106f**, **106h** and **106j**) showed antifungal property to some extents. The most astonished and discouraged result was obtained by screening the oxazolidinone derivatives. The most promising drug candidate (4*S*,5'*R*)-3'-(3-fluoro-4-morpholinophenyl)-[4,5'-bioxazolidine]-2,2'-dione (**92**) did not exhibited bioactivity against any chosen organisms. Its isomer **258** demonstrated only very week antifungal activity. This also happened in the intermediates afforded during the synthesis, beside **299** with moderate antifungal and antibacterial activity, none of them reached the original expectation. The reasons could be that the chosen bacteria are able to transport these compounds out of the cell via efflux pumps or even these molecules could not reach inside the bacteria in case to inhibit their growth.

4. Experimental Part

4.1 General

4.1.1 Chemicals

All commercial available chemicals were purchased and delivered from the chemical suppliers, for instance, Sigma Aldrich Chemie GmbH (Steinheim, Germany), Acros Organics (Geel, Belgium), Fluka Chemie GmbH (Buchs, Switzerland), abcr GmbH (Karlsruhe, Germany), TCI Deutschland GmbH (Eschborn, Germany), Merck KGaA (Darmstadt, Germany) and Carl Roth GmbH and Co. KG (Karlsruhe, Germany). They were used without any further purification. Technical grade solvents were distilled with standard method prior to usage.

4.1.2 Reaction conditions

All the flasks involved in the reactions were clean and dried over 400 ^{o}C by heating gun. All anaerobic reactions were carried out under nitrogen or argon atmosphere.

4.1.3 Thin layer chromatography

To monitor the reactions and column chromatography, analytical thin layer chromatography was used during and after the reactions. The 0.2 mm thickness and precoated silica gel TLC plates (Polygram Sil G/UV$_{254}$) were purchased from the manufacture, Macherey-Nagel, Düren, Germany. The plates were either verified under UV-light (λ = 254 nm) or colored by distinct reagents to visualize the spots (staining), including molybdophosphoric acid reagent (10 g molybdophosphoric acid and 100 mL ethanol), potassium permanganate reagent (5 g potassium permanganate, 20 g potassium carbonate, 1N sodium hydroxide and 300

mL distilled water), and ninhydrin reagent (1.5 g ninhydrin, 100 mL *n*-butanol and 3.0 mL acetic acid). After staining, the plates were warmed by heating gun for visualization.

4.1.4 Column chromatography

Silica gel 60 (0.04-0.063 mm, 230-400 mesh ASTM) was purchased by Macherey-Nagel and used for the flash column chromatography. The column was eluted with mixture of different organic solvents, such as hexane, cyclohexane, ethyl acetate, chloroform, methanol and so on, or inorganic solvent, like ammonia, as mobile phase.

4.1.5 Gas chromatography-mass spectrometry (GC-MS)

GC-MS analyses were carried out on an Agilent 7890A connected with an Agilent 5975C inert mass detector (Hewlett-Packard Company, Wilmington, USA) fitted with non polar BPX-5 (25 m, 0.22 mm i. d., 0.25 μm film, SGE Inc., Melbourne, Australia) and HP5-MS fused silica capillary column (30 m, 0.25 mm i. d., 0.25 μm film, Agilent). GC conditions were as follows: inlet pressure 77.1 kPa, He 23.3 mL min^{-1}, injection volume 1.5 μL, transfer line 300 °C, electron energy 70 eV. The operation mode was splitless (60 s valve time) and the carrier gas was He at 1.2 mL min^{-1}. The GC was programmed as follows: 5 min at 50 °C increasing with 5 °C min^{-1} to 320 °C. Retention indices (*I*) were determined from a homologous series of *n*-alkanes (C_8–C_{38}).

4.1.6 Chiral gas chromatography

Chiral gas chromatography was performed on an Agilent 7820 A fitted with CP-ChiraSil-Dex CB capillary column (25 m, 0.25 mm i. d., 0.36 μm film, Agilent) with following temperature program: 100 °C, 150 °C (1 °C/min), 245 °C (20 °C/min). The operation mode was splitless 50:1.

4.1.7 High-performance liquid chromatography (HPLC)

The separation of enantiomers **137a**, **137b**, **137c** and **137d** was done by analytical high-performance liquid chromatography using the following conditions: system: Fa. Knauer GmbH (Berlin, Germany), 2 pumps P-1 HPLC plus (max. 750 bar), oven T-1 with 2 integrated 6-Port valves, photodiode array detector PDA-1 (190-1000 nm); column: KNAUER Europher II 100-5 C18, 3µm, 16.0 mm × 250 mm; solvent: acetonitrile/water (35/65); flow rate:16.0 mL min^{-1}; pressure: 242 bar, temperature: 25 °C.

Enantiomers **137a**, **137c**, **137d** were separated by HPLC on a homochiral stationary phase using the following conditions: system: Fa. Knauer GmbH (Berlin, Germany), 2 pumps P-1 HPLC plus (max. 750 bar), oven T-1 with 2 integrated 6-Port valves, photodiode array detector PDA-1 (190-1000 nm); column: DAICEL Chiralpak IA, 5µm, 4.6 mm × 250 mm; solvent: *n*-hexane/2-propanol (98/02); flow rate:1.0 mL min^{-1}; pressure: 34 bar, temperature: 25 °C.

4.1.8 Strains and culture conditions

Geniculosporium sp. 9910 was obtained from Barbara Schulz (Braunschweig) and grown on potato–carrot medium[475] on petri dishes and incubated for 21 d at 28 °C prior to analysis. *Daldinia clavata* STMA 06094, *Daldinia childiae* MUCL 53761, *Daldinia australis* CBS 119013, *Daldinia cf. caldarium* CBS 113045, *Daldinia eschscholzii* STMA 11017, *Daldinia novae-zelandiae* STMA 05243, *Daldinia hawksworthii* STMA 13010, *Daldinia concertrica* STMA 05061, *Hypoxylon* sp.nov. JF 11167, *Hypoxylon* sp.nov. STMA 11183, *Hypoxylon macrocaipum* STMA 05121, *Hypoxylon griseobrunneum* STMA 10235, *Hypoxylon rubiginosum* STMA 03027, and *Biscogniauxia cylinderispora* STMA 12118 were acquired

from Marc Stadler (Braunschweig) and grown on YMG medium (Yeast and Malt Extract with Glucose) on petri dishes and incubated for 7 d at 28 ^{o}C prior to analysis.

4.1.9 CLSA sampling

The volatiles emitted by agar plate cultures with growing fungi were trapped on charcoal filters through the closed-loop stripping apparatus (CLSA) headspace technique. After 16 to 24 hours of collection at room temperature (20 ^{o}C) and under natural day and night light conditions, a solvent extract with dichloromethane (50 mL) of the adsorbed filter was directly analyzed by GC-MS. All headspace sampling were carried out in duplicates.

4.1.10 Spectroscopic methods

4.1.10.1 Nuclear magnetic resonance (NMR)

NMR spectra were recorded on Bruker AV I (400 MHz), AV III HD Prodigy (500 MHz) and AV III HD Cryo (700 MHz) spectrometers, and were referenced against $CDCl_3$ (δ = 7.26 ppm), C_6D_6 (δ = 7.16 ppm), CD_3OD (δ = 3.31 ppm) and DMSO-D_6 (δ = 2.50 ppm) for ^{1}H-NMR, and $CDCl_3$ (δ = 77.16 ppm), C_6D_6 (δ = 128.06 ppm), CD_3OD (δ = 49.00 ppm), DMSO-D_6 (δ = 39.52 ppm) for ^{13}C-NMR. The multiplicities are specified as follows: singlet (s), doublet (d), triplet (t), quartet (q), quintet (quin), sextet (sex), septet (sept).

4.1.10.2 Infrared spectroscopy (IR spectroscopy)

IR Spectra were measured with the method Diamant-ATR by use of Bruker Tensor 27 and an Alpha FT-IR spectrometer from Bruker. The intensities of the peaks were described as s (strong), m (medium), and w (weak).

4.1.10.3 UV-Vis

UV/Vis spectra were recorded on a Cary 100 UV/Vis spectrometer (Agilent). The wavelength of the maximal absorption was described in nm and the absorbance ε was described as $cm^{-2}mmol^{-1}$.

4.1.10.4 Optical rotatory power

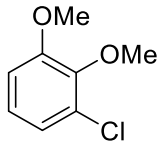
They were recorded on a P8000 Polarimeter (Krüss).

4.1.11 X-ray crystallography

All the X-ray crystallography data were obtained from slow evaporation of a concentrated solution in C_6D_6. The data collections were performed on Bruker D8-Venture and X8-KappaApexII diffractometers ((Bruker CMOS-Photon100 detector)) equipped with a low temperature device (Oxford ryostream 800er series, Oxford Cryosystems, 100K) using CuKα radiation (λ = 1.54178 Å, monochromated by a HELIOS multilayer optics). Intensities were measured by fine-slicing ω-and φ-scans and corrected for background, polarization and Lorentz effects. A semi-empirical (mulabs) absorption correction[476] was carried out on both data sets. The structures were solved by a dual-space method (SHELXT-2014)[477] and refined by full-matrix least squares on F[477] (SHELXL-2014).[478] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms at carbon were placed in calculated positions and refined isotropically using a riding model.

4.2. Synthesis

4.2.1 1-Chloro-2,3-dimethoxybenzene (105a)

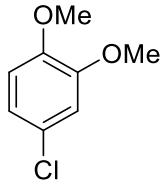


To a solution of veratrole **107** (1.38 g, 1.0 mmol) in anhydrous ether (2.0 mL), 1.6 M *n*-butyllithium in hexane (1.25 mL) was added slowly and the mixture was stirred at room temperature for 48 hours. The resulting solution of (2,3-dimethoxyphenyl)lithium was diluted with dry ether (12 mL), triethylamine (101 mg, 1.0 mmol) was added to the solution and stirred for 10 minutes. Trifluoromethanesulfonyl chloride (169 mg, 1.0 mmol) was added dropwise to the mixture. After stirring at room temperature for 1 hour, the reaction was quenched by the addition of water. The aqueous phase was extracted three times with diethyl ether. The combined extracts were dried over $MgSO_4$, concentrated in vacuo and purified by flash column chromatography on silica gel to afford **105a** (80 mg, 0.47 mmol, 47%) as pale yellow oil.

TLC (hexane/ethyl acetate = 10:1) R_f = 0.40. **IR** (ATR) $\tilde{\nu}$ = 3003 (w), 2936 (w), 2835 (w), 1582 (w), 1481 (m), 1460 (m), 1427 (m), 1295 (w), 1263 (m), 1237 (m), 1173 (w), 2253 (w), 1079 (w), 1041 (s), 1001 (s), 855 (m), 799 (w), 771 (m), 736 (m), 654 (w), 556 (w) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 280 (3.15), 273 (3.17), 231 (3.55) nm. **MS** (EI, 70 eV) *m*/*z* (%) 172 (100) $[M]^+$, 174 (33), 159 (21), 157 (65), 129 (27), 94 (22), 79 (21), 65 (30), 51 (24), 44 (21), 40 (40). **GC** (HP5-MS): *I* = 1278. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 6.96 (m, 2H, 2 x CH), 6.81 (m, 1H, CH), 3.87 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3) ppm. **^{13}C NMR** (100 MHz,

$CDCl_3$, TMS): δ = 154.0 (C), 145.5 (C), 128.4 (C), 124.3 (CH), 122.0 (CH), 110.9 (CH), 60.6 (OCH_3), 56.1 (OCH_3) ppm.

4.2.2 4-Chloro-1,2-dimethoxybenzene (105b)

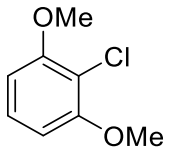


To a cooled solution (0 °C) of 3,4-dimethoxyaniline **108** (3.1 g, 20.0 mmol) in 6 M HCl (30 mL) was added a 2.5 M solution of $NaNO_2$ (1.40 g, 20.0 mmol) in H_2O (8 mL), resulting in solution A. In a separate flask $CuSO_4$ (5.8 g, 26.7 mmol) was dissolved in H_2O (25 mL) and NaCl (2.3 g, 40.0 mmol) was added. To this solution was added Na_2SO_3 (1.70 g, 13.4 mmol) in H_2O (6 mL). It was decanted from the precipitate and washed with H_2O. The precipitate was dissolved in concentrated HCl (11 mL) and cooled to 0 °C. Solution A was added dropwise and stirred for 1 h at room temperature, followed by 30 min at 100 °C. After cooling to room temperature it was extracted three times with EtOAc. The collected extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel affording **105b** (2.1 g, 12.2 mmol, 61%) as colourless oil.

TLC (hexane/ethyl acetate = 10:1) R_f = 0.22. **IR** (ATR) $\tilde{\nu}$ = 3003 (w), 2956 (w), 2908 (w), 1591 (m), 1500 (s), 1441 (m), 1402 (w), 1252 (s), 1226 (s), 1177 (m), 1131 (m), 1022 (s), 873 (m), 838 (m), 797 (m), 763 (m), 643 (m) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 283 (3.47), 235 (3.91) nm. **MS** (EI, 70 eV) *m*/*z* (%) 172 (100) $[M]^+$, 157 (64), 129 (39), 111 (15), 93 (37), 86 (8), 79 (27), 65 (35), 51 (17). **GC** (HP5-MS): *I* = 1332. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 6.87 (dd, 1H, $^4J_{H,H}$ = 2.4 Hz, $^3J_{H,H}$ = 8.5 Hz, CH), 6.84 (d, 1H, $^4J_{H,H}$ = 2.4 Hz, CH), 6.76 (d, 1H, $^3J_{H,H}$ = 8.5 Hz,

CH), 3.85 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 149.5 (C), 147.8 (C), 125.6 (C), 120.2 (CH), 112.0 (CH), 111.9 (CH), 56.02 (OCH_3), 55.98 (OCH_3) ppm.

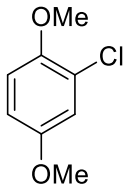
4.2.3 2-Chloro-1,3-dimethoxybenzene (105c)



To a solution of 2-chlorobenzene-1,3-diol **110** (72 mg, 0.5 mmol) in acetone (4 mL), potassium carbonate (346 mg, 2.5 mmol) was added and stirred 10 minutes. Then methyl iodide (184 mg, 1.3 mmol) was added to the reaction mixture. The reaction was stirred under reflux 16 hours. It was cooled to room temperature and extracted three times with ether. The organic phases were collected, dried over $MgSO_4$, concentrated in vacuo and purified by column chromatography on silica gel to obtain **105c** (76 mg, 0.44 mmol, 88%) as yellow solid.

TLC (hexane/ethyl acetate = 10:1) R_f = 0.38. **IR** (ATR): $\tilde{\nu}$ = 3011 cm^{-1} (w), 2966 (w), 2947 (w), 2840 (w), 1594 (m), 1472 (m), 1435 (m), 1299 (m), 1253 (m), 1191 (w), 1174 (w), 1099 (m), 1053 (m), 1025 (m), 849 (w), 764 (m), 709 (m), 654 (m), 597 (m). **UV/Vis** λ_{max} (log ε) 280 (3.08), 274 (3.10), 230 (3.79) nm. **MS** (EI, 70 eV) *m*/*z* (%) 172 (100) $[M]^+$, 173 (33), 143 (14), 142 (11), 131 (16), 129 (54), 127 (16), 114 (18), 107 (15), 79 (17), 77 (10), 65 (16), 63 (19), 51 (16). **GC** (HP5-MS): *I* = 1404. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 7.17 (t, 1H, $^3J_{H,H}$ = 8.7 Hz, CH), 6.60 (d, 2H, $^3J_{H,H}$ = 8.4 Hz, 2 x CH), 3.90 (s, 6H, 2 x OCH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 156.2 (2 x C), 127.1(CH), 110.6 (C), 104.7 (2 x CH), 56.3 (2 x OCH_3) ppm.

4.2.4 2-Chloro-1,4-dimethoxybenzene (105f)

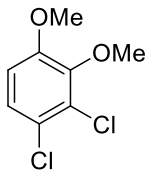


A solution of 1,4-dimethoxybenzene **111** (414 mg, 3.0 mmol) in CH_2Cl_2 was treated with $Bn(Me)_3N^+ICl_4^-$ (1.30 g, 3.0 mmol) and stirred over night at room temperature. The reaction mixture was diluted with H_2O and extracted three times with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel yielding **105f** (174 mg, 1.0 mmol, 34%) as red oil.

TLC (hexane/ethyl acetate = 20:1) R_f = 0.31. **IR** (ATR) $\tilde{\nu}$ = 3003 (w), 2948 (w), 2836 (w), 1581 (w), 1496 (s), 1461 (m), 1438 (m), 1271 (m), 1213 (s), 1180 (m), 1039 (s), 882 (m), 797 (m), 735 (s) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 294 (3.56), 230 (3.78) nm. **MS** (EI, 70 eV) *m*/*z* (%) 172 (58) $[M]^+$, 157 (100), 129 (22), 107 (12), 79 (17), 63 (14), 53 (8). **GC** (HP5-MS): *I* = 1292. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 6.95 (d, 1H, $^4J_{H,H}$ = 3.0 Hz, CH), 6.86 (d, 1H, $^4J_{H,H}$ = 3.0 Hz, $^3J_{H,H}$ = 9.0 Hz, CH), 6.76 (dd, 1H, $^4J_{H,H}$ = 3.0 Hz, $^3J_{H,H}$ = 9.0 Hz, CH), 3.84 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 153.8 (C), 149.4 (C), 123.0 (C), 116.1 (CH), 113.2 (CH), 112.8 (CH), 56.7 (OCH_3), 55.8 (OCH_3) ppm.

4.2.5 1,2-Dichloro-3,4-dimethoxybenzene (106a), 1,4-dichloro-2,3-dimethoxybenzene (106c), and 1,3-dichloro-2,4-dimethoxybenzene (106e)

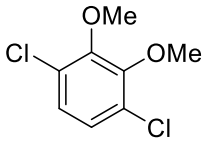
Sodium methoxide (1.08 g, 20.0 mmol) was added to a stirred solution of 1,2,3,4-tetrachlorobenzene **112** (1.08 g, 5.0 mol) in hexamethylphosphoramide (15 mL). The reaction mixture was stirred at 120 °C for 3 hours and cooled to room temperature. Then methyl iodide (1.06 g, 7.5 mmol) was added to the reaction flask and the mixture was stirred for another hour at room temperature. The mixture was poured into 2N HCl and extracted three times with diethyl ether. The organic layers were dried over $MgSO_4$ and concentrated in vacuo. From the crude product that mainly contained monosubstitution products small amounts of disubstitution products could be isolated by rigorous purification via column chromatography on silica gel. Three target compounds were obtained: **106a** (40 mg, 0.19 mmol, 4%, pale yellow oil), **106c** (100 mg, 0.48 mmol, 10%, colourless solid), and **106e** (40 mg, 0.19 mmol, 4%, colourless oil).



106a

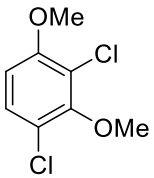
TLC (hexane/ethyl acetate = 5:1) R_f = 0.25. **IR** (ATR): $\tilde{\nu}$ = 3005 (w), 2939 (w), 2840 (w), 1579 (w), 1474 (m), 1431 (m), 1400 (m), 1291 (m), 1262 (m), 1169 (w), 1140 (w), 1043 (m), 1007 (m), 889 (w), 834 (m), 796 (m), 752 (w), 672 (m), 644 (w), 598 (w) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 288 (3.23), 283 (3.22), 230 (3.84). **MS** (EI, 70 eV): *m/z* (%) 206 (100) $[M]^+$, 208 (68), 193 (50), 191 (77), 165 (22), 163 (33), 150 (22), 148 (36), 128 (58), 127 (41), 141 (23), 115 (21), 113 (62), 99 (55), 97 (28), 85 (36), 50

(26). **GC** (HP5-MS): *I* = 1466. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 7.15 (d, 1H, $^{3}J_{H,H}$ = 9.0 Hz, CH), 6.76 (d, 1H, $^{3}J_{H,H}$ = 9.0 Hz, CH), 3.86 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 152.4 (C), 146.7 (C), 127.5 (C), 124.7 (CH), 124.5 (C), 111.0 (CH), 60.6 (OCH_3), 56.2 (OCH_3) ppm.



106c

TLC (hexane/ethyl acetate = 5:1) R_f = 0.47. **IR** (ATR): $\tilde{\nu}$ = 3003 (w), 2973 (w), 2941 (w), 2876 (w), 1579 (w), 1459 (m), 1430 (m), 1403 (m), 1239 (m), 1152 (w), 1128 (m), 1004 (s), 865 (m), 797 (m), 645 (m), 627 (m) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 274 (2.60), 231 (3.87) nm. MS (EI, 70 eV): *m/z* (%) 206 (100) $[M]^+$, 208 (74), 193 (57), 191 (82), 163 (28), 150 (39), 148 (58), 130 (24), 129 (23), 128 (63), 127 (49), 120 (25), 113 (35), 99 (48), 97 (28), 85 (38), 62 (21), 53 (31), 50 (27). **GC** (HP5-MS): *I* = 1339. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 7.07 (s, 2H, 2 x CH), 3.92 (s, 6H, 2 x OCH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 150.7 (2 x C), 127.1 (2 x C), 125.1 (2 x CH), 61.1 (2 x OCH_3) ppm.



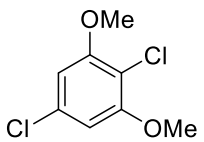
106e

TLC (hexane/ethyl acetate = 5:1) R_f = 0.56. **IR** (ATR): $\tilde{\nu}$ = 3007 (w), 2968 (w), 2941 (w), 2873 (w), 2840 (w), 1579 (w), 1466 (m), 1433 (m), 1402 (m), 1294 (m), 1270 (w), 1227 (m), 1151 (w), 1081 (s), 1008 (m), 914 (m), 794 (s), 750 (m), 686 (m), 644 (w), 576 (w) cm^{-1}. **UV-Vis** (CH_2Cl_2)

λ_{max} (log ε) 288 (3.22), 283 (3.21), 230 (3.84). **MS** (EI, 70 eV): *m/z* (%) 206 (100) $[M]^+$, 208 (67), 193 (25), 191 (38), 165 (46), 163 (74), 150 (23), 148 (36), 113 (33), 99 (25), 97 (28), 85 (21). **GC** (HP5-MS): *I* = 1487. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 7.23 (d, 1H, $^3J_{H,H}$ = 9.0 Hz, CH), 6.66 (d, 1H, $^3J_{H,H}$ = 9.0 Hz, CH), 3.90 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 155.1 (C), 153.2 (C), 127.7 (CH), 120.7 (C), 118.2 (C), 107.9 (CH), 60.6 (OCH_3), 56.5 (OCH_3) ppm.

4.2.6 2,5-Dichloro-1,3-dimethoxybenzene (106f) and 1,2-dichloro-3,5-dimethoxybenzene (106g)

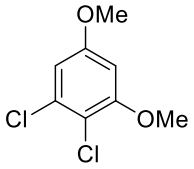
Sodium methoxide (432 mg, 8.0 mmol) was added to a stirred solution of 1,2,3,5-tetrachlorobenzene **113** (432 mg, 2.0 mmol) in hexamethylphosphoramid (15 mL) at 120 °C under the protection of nitrogen. The reaction mixture was stirred at 120 °C for 3 hours and cooled to room temperature. Then methyl iodide (426 mg, 3.0 mmol) was added to the reaction flask and stirred for another hour at room temperature. The mixture was poured into the diluted hydrochloride acid and extracted with diethyl ether. The organic layers were dried over magnesium sulfate, concentrated in vacuo and purified by flash column chromatography on silica gel to give **106f** (120 mg, 0.58 mmol, 29%, colourless solid) and **106g** (72 mg, 0.35 mmol, 18%, colourless solid).



106f

TLC (hexane/ethyl acetate = 10:1) R_f = 0.27. **IR** (ATR): $\tilde{\nu}$ = 3092 (w), 3032 (w), 2976 (w), 2942 (w), 2909 (w), 2839 (w), 1590 (m), 1567 (m), 1459 (m), 1439 (m), 1404 (m), 1315 (m), 1296 (m), 1230 (m), 1119 (m),

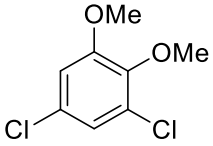
1061 (m), 914 (m), 869 (m), 820 (m), 669 (s), 633 (m), 582 (m) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 276 (3.01), 231 (3.90) nm. **MS** (EI, 70 eV): m/z (%) = 206 (100) $[M]^+$, 208 (62), 177 (15), 165 (38), 163 (65), 113 (33), 99 (32), 97 (30), 85 (15), 63 (16), 62 (22). **GC** (HP5-MS): *I* = 1556. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 6.60 (s, 2H, 2 x CH), 3.89 (s, 6H, 2 x OCH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 156.4 (2 x C), 133.0 (C), 109.3 (C), 105.5 (2 x C), 56.5 (2 x OCH_3) ppm.



106g

TLC (hexane/ethyl acetate = 10:1) R_f = 0.45. **IR** (ATR): $\tilde{\nu}$ = 3092 cm^{-1} (w), 2983 (w), 2954 (w), 2939 (w), 2837 (w), 1594 (m), 1569 (m), 1463 (m), 1429 (m), 1410 (m), 1323 (m), 1274 (m), 1214 (m), 1185 (m), 1159 (m), 1095 (m), 1030 (m), 933 (m), 857 (m), 825 (m), 782 (m), 697 (s), 658 (m), 639 (m), 621 (m). **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 289 (3.42), 229 (3.91) nm. **MS** (EI, 70 eV): m/z (%) = 206 (100) $[M]^+$, 208 (68), 177 (21), 165 (69), 163 (69), 148 (24), 141 (23), 113 (34), 99 (30), 97 (31), 85 (24), 62 (23). **GC** (HP5-MS): *I* = 1565. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 6.62 (d, 1H, $^4J_{H,H}$ = 2.7 Hz, CH), 6.42 (d, 1H, $^4J_{H,H}$ = 2.7 Hz, CH), 3.87 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 158.7 (C), 156.7 (C), 133.8 (C), 113.5 (C), 106.4 (CH), 98.6 (CH), 56.4 (OCH_3), 55.7 (OCH_3) ppm.

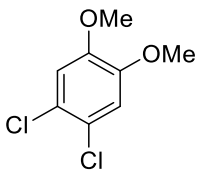
4.2.7 1,5-Dichloro-2,3-dimethoxybenzene (106b)



To a solution of 3,5-dichlorobenzene-1,2-diol **114** (54 mg, 0.3 mmol) in acetone (2.4 mL), potassium carbonate (208 mg, 1.5 mmol) was added and stirred 10 minutes. Then methyl iodide (111 mg, 0.78 mmol) was added to the reaction mixture. The reaction was stirred under reflux 16 hours. It was cooled to room temperature and extracted with ether. The organic phases were collected, dried over $MgSO_4$, concentrated in vacuo and purified by flash column chromatography on silica gel to afford **106b** (50 mg, 0.24 mmol, 80%) as colourless solid.

TLC (hexane/ethyl acetate = 10:1) R_f = 0.55. **IR** (ATR) $\tilde{\nu}$ = 3089 cm^{-1} (w), 3005 (w), 2967 (w), 2939 (w), 2831 (w), 1572 (m), 1480 (m), 1425 (m), 1399 (m), 1292 (m), 1263 (m), 1229 (m), 1171 (m), 1102 (m), 1043 (m), 999 (m), 895 (m), 830 (m), 760 (m), 718 (m), 589 (m). **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 286 (3.22), 280 (3.20), 231 (3.83) nm. **MS** (EI, 70 eV) *m*/*z* (%) 206 (84) $[M]^+$, 208 (56), 193 (65), 191 (100), 165 (27), 163 (43), 148 (23), 128 (53), 127 (53), 113 (34), 99 (46), 97 (27), 85 (31), 62 (20), 50 (28). **GC** (HP5-MS): *I* = 1426. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 6.98 (d, 1H, $^4J_{H,H}$ = 2.5 Hz, CH), 6.80 (d, 1H, $^4J_{H,H}$ = 2.5 Hz, CH), 3.86 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 154.1 (C), 144.4 (C), 129.1 (C), 128.9 (C), 121.6 (CH), 111.7 (CH), 60.8 (OCH_3), 56.3 (OCH_3) ppm.

4.2.8 1,2-Dichloro-4,5-dimethoxybenzene (106d)

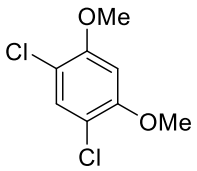


A solution of 1,2-dimethoxybenzene **107** (690 mg, 5.0 mmol) in acetic acid (15 mL) was treated with $Bn(Me)_3N^+ICl_4^-$ (4.20 g, 10.0 mmol,). It was stirred for 1 h at room temperature and then diluted with H_2O. The

mixture was extracted three times with EtOAc, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel yielding **106d** (466 mg, 2.25 mmol, 45%) as pale yellow oil.

TLC (hexane/ethyl acetate = 10:1) R_f = 0.39. **IR** (ATR) $\tilde{\nu}$ = 3004 (w), 2966 (w), 2906 (w), 2839 (w), 1594 (m), 1503 (s), 1432 (s), 1362 (m), 1337 (m), 1253 (s), 1210 (s), 1178 (s), 1131 (s), 1025 (s), 919 (s), 838 (s), 793 (s), 676 (s) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 290 (3.51), 235 (3.91) nm. **MS** (EI, 70 eV) *m/z* (%) 206 (100) $[M]^+$, 191 (62), 163 (28), 145 (15), 128 (46), 113 (33), 99 (41), 85 (12), 63 (9), 50 (12). **GC** (HP5-MS): *I* = 1505. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 6.90 (s, 2H, 2 x CH), 3.86 (s, 6H, 2 x OCH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 148.3 (2 x C), 123.4 (2 x C), 112.9 (2 x CH), 56.2 (2 x OCH_3) ppm.

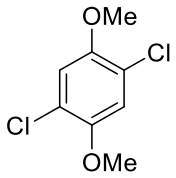
4.2.9 1,5-Dichloro-2,4-dimethoxybenzene (106h)



To a solution of 4,6-dichlorobenzene-1,3-diol **115** (54 mg, 0.3 mmol) in acetone (4 mL), potassium carbonate (208 mg, 1.5 mmol) was added and stirred 10 minutes. Then methyl iodine (111 mg, 0.78 mmol) was added to the reaction mixture. The reaction was stirred under reflux 16 hours. It was cooled to room temperature and extracted three times with ether. The organic phases were collected, dried over $MgSO_4$, concentrated in vacuo and purified by flash column chromatography on silica gel to afford 1,5-dichloro-2,4-dimethoxybenzene **106h** (49 mg, 0.24 mmol, 80%) as yellow solid.

TLC (hexane/ethyl acetate = 10:1) R_f = 0.37. **IR** (ATR) $\tilde{\nu}$ = 2976 cm^{-1} (w), 2946 (w), 2879 (w), 2847 (w), 1575 (m), 1494 (m), 1470 (m), 1455 (m), 1428 (m), 1373 (m), 1294 (m), 1231 (m), 1207 (m), 1172 (m), 1087 (m), 1055 (m), 1020 (m), 860 (m), 803 (m), 741 (m), 579 (m) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 292 (3.59), 233 (3.89) nm. **MS** (EI, 70 eV) *m*/*z* (%) 206 (84) $[M]^+$, 208 (56), 191 (23), 165 (63), 163 (100), 148 (23), 113 (23), 99 (33), 97 (29), 85 (22), 63 (15), 62 (20), 61 (17). **GC** (HP5-MS): *I* = 1545. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 7.34 (s, 1H, CH), 6.53 (s, 1H, CH), 3.90 (s, 6H, 2 x OCH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 156.4 (2 x C), 130.5 (CH), 114.0 (2 x C), 97.8 (CH), 56.5 (2 x OCH_3) ppm.

4.2.10 1,4-Dichloro-2,5-dimethoxybenzene (106j)

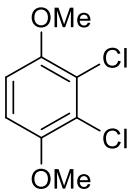


A solution of 1,4-dimethoxybenzene **111** (207 mg, 1.5 mmol) in acetic acid (6 mL) was treated with $Bn(Me)_3N^+ICl_4^-$ (1.30 g, 3.0 mmol) and stirred for 2 h at room temperature. It was diluted with water and extracted three times with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel yielding **106j** (118 mg, 0.57 mmol, 38%) as colourless oil.

TLC (hexane/ethyl acetate = 20:1) R_f = 0.30. **IR** (ATR) $\tilde{\nu}$ = 3029 (w), 2909 (w), 1501 (s), 1481 (m), 1439 (s), 1367 (m), 1279 (m), 1213 (s), 1187 (m), 1079 (s), 1025 (s), 858 (s), 775 (s) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 299 (3.69), 230 (3.90) nm. **MS** (EI, 70 eV) *m*/*z* (%) 206 (58) $[M]^+$, 191 (100), 163 (14), 141 (31), 113 (8), 97 (15), 85 (10), 53 (19). **GC**

(HP5-MS): *I* = 1448. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 6.97 (s, 2H, 2 x CH), 3.85 (s, 6H, 2 x OCH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 149.2 (2 x C), 120.9 (2 x C), 114.5 (2 x CH), 56.8 (2 x OCH_3) ppm.

4.2.11 2,3-Dichloro-1,4-dimethoxybenzene (106i)

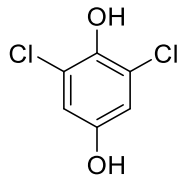


To a solution of 1,4-benzoquinone **116** (4.32 g, 40.0 mmol) in dry ether (35 mL), sulfuryl chloride (6.5 mL, 80.0 mmol) was added and stirred for 16 hours at room temperature. The mixture was cooled in an iced bath and filtered. The black solid was washed with cold ether (10 mL) and dried in high vacuo. A suspension of the solid was treated with AcOH (20 mL) and concentrated H_2SO_4 (2 mL), stirred for 24 hours at 60 ^{o}C and poured into ice. The reaction mixture was extracted three times with diethyl ether. The collected organic layers were washed with brine, dried over $MgSO_4$ and evaporated the solvents to produce brown solid (3.1 g). To this brown solid (3.1 g, 17.3 mmol) in acetone (69.2 mL), potassium carbonate (11.93 g, 86.5 mmol) was added and stirred 10 minutes. Then methyl iodide (6.39 g, 45.0 mmol) was added to the reaction mixture. The reaction was stirred under reflux 16 hours. It was cooled to room temperature and extracted three times with diethyl ether. The organic phases were collected, dried over $MgSO_4$, concentrated in vacuo and purified by flash column chromatography on silica gel to afford **106i** (1.03 g, 5.01 mmol, 13 %) as yellow solid.

TLC (hexane/ethyl acetate = 10:1) R_f = 0.42. **IR** (ATR) $\tilde{\nu}$ = 3094 (w), 2967 (w), 2946 (w), 2914 (w), 2873 (w), 2840 (w), 1591 (w), 1570 (w), 1479 (m), 1457 (m), 1406 (w), 1303 (w),1262 (m), 1192 (w), 1116 (w),

1038 (s), 900 (w), 790 (s), 711 (w), 608 (m) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 296 (3.59), 229 (3.76) nm. **MS** (EI, 70 eV) *m*/*z* (%) 206 (77) $[M]^+$, 208 (59), 195 (22), 193 (83), 192 (17), 148 (18), 143 (34), 141 (74), 115 (36), 113 (80), 99 (24), 97 (40), 87 (40), 85 (32), 63 (17), 62 (21), 61 (18). **GC** (HP5-MS): *I* = 1574. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 6.81 (s, 2H, 2 x CH), 3.86 (s, 6H, 2 x OCH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 150.3 (2 x C), 123.1 (2 x C), 110.0 (2 x CH), 56.8 (2 x OCH_3) ppm.

4.2.12 2,6-Dichlorobenzene-1,4-diol (119)

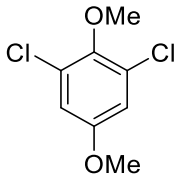


To a solution of 2,6-dichloro-*p*-hydroquinone **118** (1.0 g, 5.7 mmol) in 20 mL of ethyl acetate, 10 mL of phosphate buffer (10 mmol, Na_2HPO_4, 10 mmol KH_2PO_4, PH = 7.0) with L-ascorbic acid (4.0 g) was added. The mixture was shaken in a separated funnel for 5 mins. The mixture was extracted three times with ethyl acetate, the combined extracts were dried over $MgSO_4$ and concentrated in vacuo to afford **119** (1.0 g, 5.6 mmol, 99%) as colourless solid.

TLC (hexane/ethyl acetate = 10:1) R_f = 0.56. **IR** (ATR) $\tilde{\nu}$ = 3384 cm^{-1} (w), 3075 (w), 2900 (w), 2517 (m), 2415 (m), 2077(w), 1791 (m), 1746 (w), 1687 (w), 1614 (w), 1577 (m), 1477 (s), 1430 (m), 1344 (m), 1275 (w), 1213 (m), 1112 (m), 1095 (m), 971 (s), 947 (s), 844 (m), 803 (s), 700 (w), 599 (w) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 351(1.38), 296 (3.58), 229 (3.49) nm. **MS** (EI, 70 eV) *m*/*z* (%) 180 (63) $[M+1H]^+$, 178 (92), 114 (18), 113 (21), 88 (34), 87 (41), 86 (47), 85 (31), 79 (53), 62 (30), 61 (29), 60 (68), 63 (16), 53 (100), 51 (54), 50 (43), 49 (22). **^{1}H NMR** (400 MHz,

$CDCl_3$, TMS): δ = 6.72 (s, 2H, 2 x CH), 4.86 (s, 2H, 2 x OH) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 151.9 (C), 143.5 (C), 123.9 (2 x C), 116.4 (2 x CH) ppm.

4.2.13 1,3-Dichloro-2,5-dimethoxybenzene (106k)

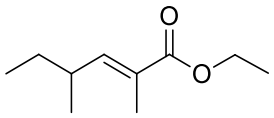


To a solution of 2,6-dichlorobenzene-1,4-diol **118** (90 mg, 0.5 mmol,) in acetone (4 mL), potassium carbonate (691 mg, 5.0 mmol) was added and stirred 10 minutes. Then methyl iodine (185 mg, 1.3 mmol) was added to the reaction mixture. The reaction was stirred under reflux 16 hours. It was cooled to room temperature and extracted three times with diethyl ether. The organic phases were collected, dried over $MgSO_4$, concentrated in vacuo and purified by flash column chromatography on silica gel to afford 1,3-dichloro-2,5-dimethoxybenzene **106k** (49 mg, 0.24 mmol, 48%) as colourless solid.

TLC (hexane/ethyl acetate = 10:1) R_f = 0.56. **IR** (ATR) $\tilde{\nu}$ = 3087 cm^{-1} (w), 2992 (w), 2949 (w), 2903 (w), 2835 (w), 1704 (w), 1663(w), 1610 (m), 1594 (m), 1559 (m), 1480 (s), 1420 (m), 1403 (m), 1306 (m), 1257 (m), 1222 (s), 1175 (m), 1074 (m), 1042 (s), 984 (s), 909 (w), 909 (m), 850 (m), 831 (m), 804 (s), 761 (s), 717 (m), 606 (m) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 292 (3.56), 287 (3.45), 230 (3.78) nm. **MS** (EI, 70 eV) *m*/*z* (%) 206 (56) [M]$^+$, 208 (39), 195 (18), 193 (78), 192 (16), 191 (100), 165 (22), 163 (32), 163 (32), 113 (25), 99 (32), 97 (24), 63 (16), 62 (22), 62 (22), 53 (26). **GC** (HP5-MS): *I* = 1443. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 6.84 (s, 2H, 2 x CH), 3.84 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 155.7 (C), 146.2 (C), 129.5 (2 x C),

114.5 (2 x CH), 60.8 (OCH_3), 55.9 (OCH_3) ppm.

4.2.14 (*E*)-Ethyl 2,4-dimethylhex-2-enoate (142)



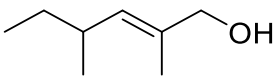
To a solution of LiCl (1.02 g, 24.0 mmol), DBU (3.04 g, 20.0 mmol) and ethyl 2-(diethoxyphosphoryl)propanoate (5.72 g, 24 mmol) in acetonitrile (240 mL), 2-methylbutanal (1.72 g, 20.0 mmol) was added and stirred at room temperature for one hour. Then, it was quenched with distilled water, extracted with ether. The collected organic phases were dried over $MgSO_4$, concentrated in vacuo and purified by flash column chromatography on silica gel to afford (*E*)-ethyl 2,4-dimethylhex-2-enoate **142** (1.3 g, 7.67 mmol, 38%) and ethyl (*Z*)-2,4-dimethylhex-2-enoate (15 mg, 0.088 mmol, 1%) as colourless oil.

Analytical data for (*E*)-**142**: **TLC** (hexane/ethyl acetate = 20:1) R_f = 0.31. **IR** (ATR) $\tilde{\nu}$ = 2962 (m), 2930 (m), 2874 (m), 1709 (w), 1650 (m), 1458 (m), 1367 (m), 1316 (m), 1269 (w), 1235 (w), 1152 (w), 1033 (m), 995 (m), 924 (m), 871 (m), 776 (s), 748 (w), 532 (s) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 231 (4.71) nm. **MS** (EI, 70 eV): *m/z* (%) = 170 (60) $[M]^+$, 125 (70), 123 (21), 113 (87), 109 (57), 102 (28), 97 (41), 96 (70), 95 (89), 87 (30), 85 (28), 81 (43), 73 (20), 69 (44), 67 (89), 56 (33), 55 (100), 53 (44), 43 (72), 41 (97), 39 (55). **GC** (BPX-5): *I* = 1152. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 6.53 (dq, $^4J_{H,H}$ = 1.4 Hz,$^3J_{H,H}$ = 10.0 Hz, 1H, CH), 4.19 (q, $^3J_{H,H}$ = 7.1 Hz, 2H, CH_2), 2.48-2.32 (m, 1H, CH), 1.84 (d, $^4J_{H,H}$ = 1.4 Hz, 3H, CH_3), 1.50-1.34 (m, 2H, CH_2), 1.30 (t, $^3J_{H,H}$ = 7.1 Hz, 3H, CH_3), 1.00 (d, $^3J_{H,H}$ = 6.7 Hz, 3H, CH_3), 0.86 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 168.5 (C), 147.8 (CH), 126.5 (C),

60.4 (CH_2), 34.9 (CH), 29.6 (CH_2), 19.6 (CH_3), 14.3 (CH_3), 12.5 (CH_3), 11.9 (CH_3) ppm.

Analytical data for (*Z*)-**142**: **TLC** (hexane/ethyl acetate = 20:1) R_f = 0.29. **IR** (ATR) $\tilde{\nu}$ = 2962 (m), 2930 (m), 2873 (m), 1715 (w), 1646 (m), 1456 (m), 1372 (m), 1326 (m), 1262 (w), 1221 (w), 1171 (w), 1096 (m), 1044 (m), 1023 (m), 971 (m), 889 (s), 771 (m), 594 (s) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 229 (4.71) nm. **MS** (EI, 70 eV): *m/z* (%) = 170 (56) $[M]^+$, 125 (72), 123 (21), 113 (69), 109 (61), 102 (25), 97 (34), 96 (59), 87 (25), 81 (40), 69 (39), 67 (81), 56 (32), 55 (79), 53 (41), 43 (71), 41 (87), 39 (50). **GC** (BPX-5): *I* = 1081. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 6.53 (dq, $^4J_{H,H}$ = 1.5 Hz,$^3J_{H,H}$ = 10.1 Hz, 1H, CH), 4.18 (q, $^3J_{H,H}$ = 7.1 Hz, 2H, CH_2), 3.04-2.93 (m, 1H, CH), 1.88 (d, $^4J_{H,H}$ = 1.5 Hz, 3H, CH_3), 1.38-1.21 (m, 2H, CH_2), 1.29 (t, $^3J_{H,H}$ = 7.1 Hz, 3H, CH_3), 0.96 (d, $^3J_{H,H}$ = 6.6 Hz, 3H, CH_3), 0.84 (t, $^3J_{H,H}$ = 7.5 Hz, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 168.5 (C), 148.3 (CH), 126.1 (C), 60.0 (CH_2), 35.0 (CH), 30.1 (CH_2), 20.8 (CH_3), 20.2 (CH_3), 14.2 (CH_3), 11.8 (CH_3), ppm.

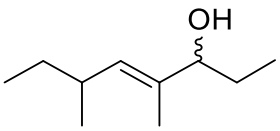
4.2.15 (*E*)-2,4-Dimethylhex-2-en-1-ol (143)



To a solution of (*E*)-ethyl 2,4-dimethylhex-2-enoate **142** (1.16 g, 6.8 mmol) in anhydrous dichloromethane (27.2 mL), DIBAL-H (13.6 mL, 13.6 mmol, 1M in Hexane) was added dropwise at -78 °C and stirred at - 78 °C overnight. Then, saturated NH_4Cl aqueous solution was added and allowed to warm to room temperature. The reaction mixture was filtered through Celite®, extracted with dichloromethane, dried over $MgSO_4$, concentrated in vacuo and purified by flash column chromatography on silica gel to produce **143** (481 mg, 3.75 mmol, 55%) as colourless oil.

TLC (hexane/ethyl acetate = 5:1) R_f = 0.32. **IR** (ATR) $\tilde{\nu}$ = 3321 (m), 2960 (m), 2922 (m), 2872 (m), 1454 (m), 1377 (m), 1260 (m), 1237 (w), 1091 (w), 1003 (s), 949 (m), 863 (m), 808 (m), 771 (w), 602 (w) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 231 (3.24) nm. **MS** (EI, 70 eV): *m/z* (%) = 128 (7) $[M]^+$, 97 (53), 95 (23), 83 (7), 81 (13), 79 (7), 73 (77), 71 (32), 69 (18), 67 (13), 58 (10), 57 (17), 56 (10), 55 (52), 44 (10), 43 (100), 41 (25), 40 (13), 39 (11). **GC** (HP-5MS): *I* = 996. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 5.17 (ddq, $^4J_{H,H}$ = 1.3 Hz, $^3J_{H,H}$ = 9.5, 2.7 Hz, 1H, CH), 4.00 (d, $^4J_{H,H}$ = 1.2 Hz, 2H, CH_2), 2.37-2.21 (m, 1H, CH), 1.67 (d, $^4J_{H,H}$ = 1.4 Hz, 3H, CH_3), 1.46 (s, 1H, OH), 1.42-1.15 (m, 2H, CH_2), 0.93 (d, $^3J_{H,H}$ = 6.7 Hz, 3H, CH_3), 0.84 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 133.4 (CH), 132.7 (C), 69.0 (CH_2), 33.7 (CH), 30.9 (CH_2), 30.2 (CH_3), 13.8 (CH_3), 11.9 (CH_3) ppm.

4.2.16 (*E*)-4,6-Dimethyloct-4-en-3-ol (144)



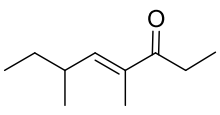
To a solution of (*E*)-2,4-dimethylhex-2-en-1-ol **143** (481 mg, 3.75 mmol) in anhydrous dichloromethane (7.5 mL), pyridium chlorochromate (968 mg, 4.5 mmol) was added at room temperature and stirred overnight at this temperature. The reaction mixture was filtered through silica gel, concentrated in vacuo and used for next step without purification.

To a solution of allylic aldehyde (3.75 mmol) in anhydrous diethyl ether (7.5 mL), prepared EtMgBr (7.5 mL, 7.50 mmol, 1M in Et_2O) was added dropwise at 0 °C and stirred at 0 °C for two hours. Then, distilled water was put into the reaction mixture slowly, extracted with Et_2O, dried over $MgSO_4$, concentrated in vacuo and purified by flash column

chromatography on silica gel to obtain **144** (328 mg, 2.10 mmol, 56%, *dr* 1:1) as colourless oil.

Analytical data for mixture of diastereomers: **TLC** (hexane/ethyl acetate = 10:1) R_f = 0.38. **IR** (ATR) $\tilde{\nu}$ = 3359 (m), 2960 (s), 2930 (m), 2873 (m), 1457 (m), 1376 (m), 1315 (w), 1261 (w), 1237 (w), 1093 (m), 1050 (m), 991 (s), 964 (m), 890 (m), 866 (m), 830 (w), 776 (m), 620 (w), 546 (m) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 230 (3.19) nm. **MS** (EI, 70 eV): **A**: *m/z* (%) = 156 (3) $[M]^+$, 128 (43), 111 (10), 110 (95), 100 (100), 98 (14), 96 (13), 92 (8), 86 (29), 84 (32), 82 (17), 78 (13), 77 (11), 73 (14), 72 (82), 70 (45), 68 (28), 58 (91), 56 (41), 44 (90), 42 (29), 41 (30). **B**: *m/z* (%) = 156 (2) $[M]^+$, 128 (45), 110 (11), 109 (96), 99 (100), 97 (12), 85 (20), 83 (34), 81 (20), 73 (11), 71 (76), 69 (40), 67 (27), 57 (80), 55 (35), 44 (34), 43 (47), 41 (28), 40 (26). **GC** (HP-5MS): *I* = 1111 (**A**) and 1115 (**B**). **^{1}H NMR** (500 MHz, $CDCl_3$, TMS): δ = **A**: 5.15-5.09 (m, 1H, CH), 3.89 (t, $^3J_{H,H}$ = 6.8 Hz, 1H, CH), 2.32-2.22 (m, 1H, CH), 1.58 (d, $^4J_{H,H}$ = 1.4 Hz, 3H, CH_3), 1.57-1.47 (m, 2H, CH_2), 1.39-1.14 (m, 2H, CH_2), 0.92 (dd, $^3J_{H,H}$ = 12.0, 6.7 Hz, 3H, CH_3), 0.86-0.79 (m, 6H, 2 x CH_3) ppm. **B**: 5.15-5.09 (m, 1H, CH), 3.89 (t, $^3J_{H,H}$ = 6.8 Hz, 1H, CH), 2.32-2.22 (m, 1H, CH), 1.58 (d, $^4J_{H,H}$ = 1.4 Hz, 3H, CH_3), 1.57-1.47 (m, 2H, CH_2), 1.39-1.14 (m, 2H, CH_2), 0.92 (dd, $^3J_{H,H}$ = 12.0, 6.7 Hz, 3H, CH_3), 0.86-0.79 (m, 6H, 2 x CH_3) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$, TMS): δ = **A**: 135.6 (C), 133.3 (CH), 79.5 (CH), 33.8 (CH), 30.4 (CH_2), 27.8 (CH_2), 20.9 (CH_3), 12.1 (CH_3), 11.6 (CH_3), 10.1 (CH_3) ppm. **B:** 135.5 (C), 133.9 (CH), 80.0 (CH), 33.9 (CH), 30.5 (CH_2), 27.7 (CH_2), 20.8 (CH_3), 12.1 (CH_3), 11.4 (CH_3), 10.2 (CH_3) ppm.

4.2.17 (*E*)-4,6-Dimethyl-4octen-3-one (133)



To a solution of **144** (312 mg, 2.0 mmol) in anhydrous dichloromethane (4.0 mL), pyridinium chlorochromate (520 mg, 2.4 mmol) was added and stirred overnight at room temperature. The reaction mixture was filtered over silica gel, concentrated in vacuo and purified by flash column chromatography on silica gel to afford (*E*)-4,6-dimethyl-4octen-3-one **133** (114 mg, 0.74 mmol, 37%) as colourless oil.

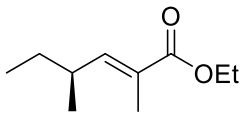
TLC (hexane/ethyl acetate = 10:1) R_f = 0.53. **IR** (ATR) $\tilde{\nu}$ = 2962 (m), 2931 (m), 2875 (m), 1670 (s), 1458 (m), 1375 (m), 1341 (m), 1262 (m), 1227 (m), 1132 (m), 1085 (m), 1051 (m), 1015 (m), 991 (m), 896 (m), 800 (m), 581 (w) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 308 (2.79), 233 (5.05) nm. **MS** (EI, 70 eV): A: *m/z* (%) = 154 (33) $[M]^+$, 155 (4), 126 (9), 125 (100), 106 (8), 97 (11), 96 (4), 84 (6), 81 (4), 69 (17), 67 (5), 57 (11), 56 (3), 55 (43), 53 (3), 43 (13), 41 (9), 40 (5). **GC** (HP-5MS): *I* = 1140. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 6.37 (dq, $^3J_{H,H}$ = 9.7 Hz, $^4J_{H,H}$ = 1.4 Hz, 1H, CH), 2.69 (q, $^3J_{H,H}$ = 7.3 Hz, 2H, CH_2), 2.56-2.40 (m, 1H, CH), 1.79 (d, $^4J_{H,H}$ = 1.3 Hz, 3H, CH_3), 1.53-1.24 (m, 2H, CH_2), 1.16-0.96 (m, 6H, 2 x CH_3), 0.87 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 202.9 (C), 47.7 (CH), 135.6 (C), 35.1 (CH), 30.4 (CH_2), 29.7 (CH_2), 19.69 (CH_3), 11.89 (CH_3), 11.6 (CH_3), 8.9 (CH_3) ppm.

4.2.18 Preparation of stereoisomers of 137

To a solution of diisopropylamine (2.02 g, 20.0 mmol) in anhydrous THF (50 mL) was added n-butyllithium (12.5 mL, 1.6 M in hexane, 20.0 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 hour and subsequently cooled to -78 °C. Pentan-3-one (1.72 g, 20.0 mmol) in anhydrous THF (10 mL) was added slowly at -78 °C and the mixture was stirred at this temperature for another hour, followed by the addition of 2-methylbutanal (1.72 g, 20.0 mmol) in anhydrous THF (10 mL). Stirring at -78 °C was continued for one more hour. The mixture was

warmed to room temperature, hydrolysed with saturated ammonium chloride solution and extracted three times with diethyl ether. The collected organic phases were dried over $MgSO_4$ and concentrated under reduced pressure to yield 5-hydroxy-4,6-dimethyloctan-3-one (2.34 g, 13.6 mmol, 68%) as a mixture of eight stereoisomers. Spectroscopic data are given below for the enantioselectively synthesised or chromatographically purified stereoisomers.

4.2.19 (2*E,4Z*)-Ethyl 2,4-dimethylhex-2-enoate (148)

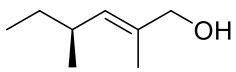


To a solution of oxalyl chloride (3.81 g, 30.0 mmol) in anhydrous dichloromethane (16.6 mL), dimethyl sulfoxide (4.88 g, 62.5 mmol) was added dropwise at -78 °C. After stirring 5 minutes, (*S*)-(-)-2-methylbutanol (2.20 g, 25.0 mmol) in anhydrous dichloromethane (6.9 mL) was added to the reaction mixture and stirred for another 30 minutes at -78 °C. Then, triethylamine (12.65 g, 150 mmol) was added to the solution and it was warmed to room temperature, (ethoxycarbonylethylidiene) triphenyl phosphorane (10.85 g, 30 mmol) was put into the flask and stirred under reflux for 24 hours. The reaction mixture was washed with distilled water, extracted with dichloromethane, dried over $MgSO_4$, concentrated in vacuo and purified by flash column chromatography on silica gel to afford ethyl (2*E*,4*S*)-2,4-dimethylhex-2-enoate **148** as colourless oil (2.27 g, 13.34 mmol, 53%) and small amount of ethyl (2*Z*,4*S*)-2,4-dimethylhex-2-enoate (0.13 g, 0.76 mmol, 3%) as colourless oils.

TLC (hexane/ethyl acetate = 10:1) R_f = 0.44. **IR** (ATR) $\tilde{\nu}$ = 2962 (w), 2930 (w), 2874 (w), 1709 (s), 1650 (w), 1458 (w), 1367 (w), 1316 (w),

1269 (m), 1235 (s), 1152 (m), 1095 (m), 1033 (w), 995 (w), 924 (w), 871 (w), 776 (w), 748 (s), 533 (w) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 231 (4.80) nm. **MS** (EI, 70 eV): *m/z* (%) = 170 (34) $[M]^+$, 125 (39), 123 (37), 113 (48), 109 (31), 96 (46), 95 (51), 81 (23), 69 (24), 68 (21), 67 (90), 65 (24), 55 (62), 53 (58), 43 (64), 42 (23), 41 (100), 40 (20), 39 (83). **GC** (BPX-5): *I* = 1156. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 5.57 (dq, $^4J_{H,H}$ = 1.4 Hz, $^3J_{H,H}$ = 10.0 Hz, 1H, CH), 4.12 (q, $^3J_{H,H}$ = 7.1 Hz, 2H, CH_2), 2.96-2.87 (m, 1H, CH), 1.82 (d, $^4J_{H,H}$ = 1.4 Hz, 3H, CH_3), 1.27-1.20 (m, 2H, CH_2), 1.23 (t, $^3J_{H,H}$ = 7.1 Hz, 3H, CH_3), 0.89 (d, $^3J_{H,H}$ = 6.6 Hz, 3H, CH_3), 0.78 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 168.4 (C), 148.3 (CH), 126.0 (C), 60.0 (CH_2), 35.0 (CH), 30.2 (CH_2), 20.8 (CH_3), 20.2 (CH_3), 14.2 (CH_3), 11.8 (CH_3) ppm. **Optical rotation**: $[\alpha]_D^{20}$= +28.4 (c 0.1, ethanol).

4.2.20 (2*E*,4*S*)-2,4-Dimethylhex-2-en-1-ol (149)

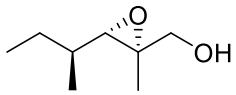


To a solution of (2*E*,4*S*)-ethyl 2,4-dimethylhex-2-enoate (6.91 g, 40.7 mmol) in anhydrous dichloromethane (397.2 mL), diisobutylammonium hydrid (1.2 M in toluene, 165.5 mL, 198.6 mmol) was added dropwise at -78 °C and stirred at this temperature overnight. A solution of saturated NH_4Cl was added and the solution was warmed to room temperature. The white sediment was filtered and the reaction mixture was extracted with dichloromethane. The collected organic layers were dried over $MgSO_4$, concentrated in vacuo and purified by flash column chromatography on silica gel to achieve **149** as colourless oil (4.04 g, 31.5 mmol, 77%).

TLC (hexane/ethyl acetate = 5:1) R_f = 0.31. **IR** (ATR) $\tilde{\nu}$ = 3319 (w), 2960 (m), 2922 (m) 2872 (m), 1454 (m), 1376 (w), 1236 (w), 1115 (w), 1003

(s), 949 (w), 863 (w), 811 (w), 711 (w), 601 (w) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 227 (2.81), 221 (2.31) nm. **MS** (EI, 70 eV): *m/z* (%) = 110 (1) $[M\text{-}H_2O]^+$, 87 (7), 71 (6), 70 (6), 69 (22), 67 (7), 59 (7), 58 (29), 57 (67), 56 (10), 55 (51), 53 (15), 51 (10), 45 (39), 43 (74), 42 (25), 41 (67), 40 (12), 39 (100), 38 (7). **GC** (BPX-5): *I* = 1068. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 4.98 (dt, $^4J_{H,H}$ = 0.5 Hz, $^3J_{H,H}$ = 9.9 Hz, 1H, CH), 4.09-4.01 (m, 2H, CH_2), 2.30-2.19 (m, 1H, CH), 1.73 (d, $^4J_{H,H}$ = 1.5 Hz, 3H, CH_3), 1.99-2.04 (m, 1H, OH), 1.31-1.06 (m, 2H, CH_2), 0.86 (d, $^3J_{H,H}$ = 6.6 Hz, 3H, CH_3), 0.75 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 135.0 (C), 132.9 (CH), 61.9 (CH_2), 33.8 (CH), 30.4 (CH_2), 21.3 (CH_3), 21.2 (CH_3), 11.9 (CH_3) ppm. **Optical rotation**: $[\alpha]_D^{20}$ = +10.4 (c 0.1, ethanol).

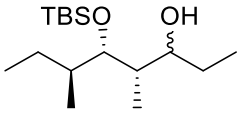
4.2.21 (2*S*,3*S*,4*S*)-2,3-Epoxy-2,4-dimethylhexan-1-ol (150a)



In a round-bottom flask, molecular sieves (2.34 g, 4Å, 1.0 g/10 mmol) and dry dichloromethane (117 mL) were settled and cooled to -10 °C. Then, (+)-diethyl L-tartrate (579 mg, 2.81 mmol), titanium isopropoxide (665 mg, 2.34 mmol) and cumene hydroperoxide (4.45, 80% in cumene, 23.4 mmol) were added and stirred for 10 minutes at -10 °C. The reaction mixture was subsequently cooled to -23 °C, alcohol **149** (3.00 g, 23.4 mmol) in anhydrous dichloromethane (8.30 mL, 2M) was added dropwise and stirred overnight at -23 °C. Distilled water was added to the solution and stirred for another 30 minutes. It was filtered over Celite®, dried over $MgSO_4$, concentrated in vacuo and purified by flash column chromatography on silica gel to achieve **150a** as colourless oil (2.71 g, 18.8 mmol, 80%, *dr* > 10:1 by GC/MS analysis).

TLC (hexane/ethyl acetate = 1:1) R_f = 0.47. **IR** (ATR) $\tilde{\nu}$ = 2960 (m), 2932 (m), 2881 (w), 2859 (w), 1708 (m), 1462 (w), 1383 (w), 1254 (w), 1182 (w), 1057 (m), 1022 (m), 962 (w), 940 (m), 835 (s), 810 (m), 773 (s), 674 (m) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 231 (2.04) nm. **MS** (EI, 70 eV): *m/z* (%) = 126 (0.5) $[M\text{-}H_2O]^+$, 111 (1), 97 (4), 87 (11), 75 (13), 74 (9), 71 (10), 70 (16), 69 (33), 67 (5), 59 (22), 58 (58), 57 (58), 56 (7), 55 (74), 53 (10), 51 (6), 45 (46), 43 (100), 42 (20), 41 (68), 40 (8), 39 (52), 38 (5). **GC** (BPX-5): *I* = 1106. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 3.68 (dd, $^4J_{H,H}$ = 4.7 Hz, $^3J_{H,H}$ = 12.2 Hz, 1H, CH_2), 3.57 (dd, $^3J_{H,H}$ = 8.2, 12.2 Hz, 1H, CH_2), 2.77 (d, $^3J_{H,H}$ = 8.9 Hz, 1H, CH), 2.05-1.99 (m, 1H, OH), 1.67-1.55 (m, 1H, CH), 1.40-1.31 (m, 2H, CH_2), 1.30 (s, 3H, CH_3), 0.97 (t, $^3J_{H,H}$ = 7.3 Hz, 3H, CH_3), 0.9 (d, $^3J_{H,H}$ = 6.6 Hz, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 65.6 (CH_2), 64.9 (CH), 60.3 (C), 34.1 (CH), 27.9 (CH_2), 15.6 (CH_3), 14.2 (CH_3), 11.3 (CH_3) ppm. **Optical rotation**: $[\alpha]_D^{20}$ = +12.9 (c 0.1, ethanol).

4.2.22 (4*S*,5*S*,6*S*)-5-((*tert*-Butyldimethylsilyl)oxy)-4,6-dimethyloctan-3-ol (152a)



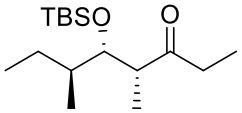
To a solution of epoxyl alcohol **150a** (2.11 g, 14.6 mmol), 2.92 g of 4Å molecular sieves and diisopropylethylamine (2.54 g, 19.7 mmol) in anhydrous dichloromethane (43.8 mL), *tert*-butyldimethylsilyl trifluoromethanesulfonate (5.02 g, 19.0 mmol) was added dropwise at -42 °C and stirred for 2 hours at this temperature. It was poured on diethyl ether and 100 mL of PH 5.5 phosphate buffer was added to the reaction mixture. The aqueous phase was extracted with ether. The collected organic layers were washed with distilled water, 5% $NaHCO_3$ and birne,

dried over $MgSO_4$ and concentrated in vacuo to generate colourless oil and used without further purification.

To the reaction mixture in anhydrous diethyl ether (38.2 mL), prepared EtMgBr (38.2 mL, 38.2 mmol, 1.0 mol/L) was added dropwise at 0 °C and stirred for 2 hours at this temperature. Then, it was washed with distilled water, extracted with ether, dried over $MgSO_4$, concentrated in vacuo and purified by flash column chromatography on silica gel to obtain **152a** (2.03 g, 7.0 mmol, 48%, colourless oil) as a mixture of diastereomers (*dr* = 55:45).

TLC (hexane/ethyl acetate = 20:1) R_f = 0.21. **IR** (ATR) $\tilde{\nu}$ = 3462 (w), 2959 (m), 2932 (m), 2880 (w), 2858 (w), 1463 (w), 1382 (w), 1253 (m), 1109 (w), 1045 (m), 1002 (m), 971 (m), 833 (s), 772 (s), 675 (m) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 286 (2.54), 232 (4.35) nm. **MS** (EI, 70 eV, major diastereomer): *m/z* (%) = 231 (17) $[M\text{-}C(CH_3)_3]^+$, 230 (7), 213 (6), 202 (12), 201 (57), 175 (8), 174 (15), 173 (100), 162 (5), 161 (42), 147 (20), 145 (11), 143 (11), 133 (13), 119 (9), 115 (32), 105 (18), 83 (10), 77 (6), 76 (7), 75 (91), 74 (6), 73 (58), 69 (14), 59 (8), 57 (10), 55 (8), 43 (10), 41 (9). **GC** (HP-5): *I* = 1615 (major diastereomer) and *I* = 1594 (minor diastereomer). **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 3.73 (dd, $^4J_{H,H}$ = 3.0 Hz, $^3J_{H,H}$ = 4.7 Hz, 1H, CH), 3.53 (ddd, $^4J_{H,H}$ = 3.3 Hz, $^3J_{H,H}$ = 6.4 Hz, 1H, CH), 2.01 (br s, 1H, OH), 1.71-1.53 and 1.18-1.04 (m, 6H, 2xCH, 2xCH_2), 0.11-0.05 (n, 6H, 2xCH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS, major diastereomer): δ = 79.0 (CH), 76.3 (CH), 40.1 (CH), 39.7 (CH), 28.1 (CH_2), 26.1 (3xCH_3), 25.8 (CH_2), 18.4 (C), 14.8 (CH_3), 12.5 (CH_3), 10.5 (CH_3), 8.7 (CH_3), -3.4 (CH_3), -4.2 (CH_3) ppm. **Optical rotation**: $[\alpha]_D^{20}$= +12.9 (c 0.1, ethanol).

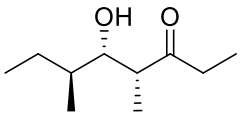
4.2.23 (4*R*,5*S*,6*S*)-5-((*tert*-Butyldimethylsilyl)oxy)-4,6-dimethyloctan-3-one (153a)



To a solution of alcohol **152a** (1.32 g, 4.56 mmol) in anhydrous dichloromethane (9.0 mL), pyridinium chlorochromate (1.32 g, 9.12 mmol) was added and stirred overnight at room temperature. The reaction mixture was filtered over silica gel, concentrated in vacuo and purified by flash column chromatography on silica gel to produce **153a** as colourless oils (1.16 g, 4.03 mmol, 88%).

TLC (hexane/ethyl acetate = 10:1) R_f = 0.55. **IR** (ATR) $\tilde{\nu}$ = 2959 (m), 2933 (m), 2880 (w), 2858 (w), 1713 (m), 1674 (w), 1461 (m), 1380 (w), 1254 (m), 1099 (m), 1051 (m), 1006 (m), 973 (m), 866 (m), 834 (s), 803 (m), 773 (s), 773 (m) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 237 (2.40) nm. **MS** (EI, 70 eV): *m/z* (%) = 286 (1) $[M]^+$, 230 (18), 229 (100), 206 (16), 201 (13), 173 (10), 145 (26), 143 (76), 115 (16), 75 (65), 73 (24), 56 (24), 44 (16), 40 (13). **GC** (HP-5MS): *I* = 1581. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 3.89 (dd, $^4J_{H,H}$ = 3.9 Hz, $^3J_{H,H}$ = 5.8 Hz, 1H, CH), 2.70 (m, 1H, CH), 2.61-2.43 (m, 2H, CH_2), 1.43-1.38 (m, 2H, CH_2), 1.07 (dd, $^4J_{H,H}$ = 2.6 Hz, $^3J_{H,H}$ = 7.0 Hz, 3H, CH_3), 1.04 (t, $^3J_{H,H}$ = 7.3 Hz, 3H, CH_3), 1.09-1.02 (m, 1H, CH), 0.89 (s, 12H, 4 x CH_3), 0.90-0.87 (m, 3H, CH_3), 0.04 (d, $^3J_{H,H}$ = 16.9 Hz, 6H, 2 x CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 214.5 (C), 76.7 (CH), 49.5 (CH), 41.0 (CH), 35.6 (CH_2), 26.3 (3 x CH_3), 25.2 (CH_2), 18.7 (C), 15.9 (CH_3), 13.9 (CH_3), 12.5 (CH_3), 8.0 (CH_3), -3.8 (CH_3), -3.9 (CH_3) ppm. **Optical rotation**: $[\alpha]_D^{20}$ = –10.8 (c 0.37, ethanol).

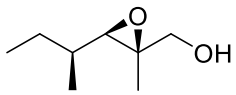
4.2.24 (4*R*,5*S*,6*S*)-5-Hydroxy-4,6-dimethyloctan-3-one (137a)



To a solution of **153a** (178 mg, 0.62 mmol) in anhydrous THF (5 mL), hydrogen fluoride pyridine (5.2 mL, 70% HF) was added carefully at 0 °C and stirred at this temperature for 1 hour. It was quenched with aqueous $NaHCO_3$ solution until no further effervescence and neutral PH. The reaction mixture was extracted with diethyl ether, dried over $MgSO_4$, concentrated in vacuo and purified by column chromatography on silica gel to yield **137a** as colourless oils (48 mg, 0.31 mmol, 50%).

TLC (hexane/ethyl acetate = 5:1) R_f = 0.33. **IR** (ATR) $\tilde{\nu}$ = 3490 (w), 2996 (m), 2937 (m), 2878 (m), 1701 (s), 1459 (m), 1410 (w), 1379 (m), 1299 (w), 1237 (w), 1149 (w), 1105 (w), 967 (m), 846 (w), 8023 (w), 775 (w) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 229 (3.26), 201 (3.74) nm. **MS** (EI, 70 eV): *m/z* (%) = 154 (6) $[M\text{-}H_2O]^+$, 125 (9), 115 (28), 98 (4), 97 (23), 87 (14), 86 (68), 85 (5), 83 (4), 71 (5), 70 (6), 69 (29), 58 (11), 57 (100), 56 (5), 55 (9), 45 (13), 43 (7), 41 (15), 39 (3). **GC** (HP-5MS): *I* = 1240. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 3.62 (dt, $^4J_{H,H}$ = 2.6 Hz, $^3J_{H,H}$ = 8.9 Hz, 1H, CH), 2.88 (d, $^4J_{H,H}$ = 3.0 Hz, 1H, OH), 2.74 (dq, $^4J_{H,H}$ = 2.5 Hz, $^3J_{H,H}$ = 7.2 Hz, 1H, CH), 2.63-2.44 (m, 2H, CH_2), 1.84-1.3 (m, 2H, CH_2), 1.20-1.14 (m, 1H, CH), 1.11 (d, $^3J_{H,H}$ = 7.2 Hz, 3H, CH_3), 1.07 (t, $^3J_{H,H}$ = 7.2 Hz, 3H, CH_3), 0.91(t, $^3J_{H,H}$ = 7.4 Hz, 3H, CH_3), 0.81 (d, $^3J_{H,H}$ = 6.8 Hz, 3H, CH_3) ppm. **^{13}C NMR (**100 MHz, $CDCl_3$, TMS): δ = 217.1 (C), 74.4 (CH), 47.0 (CH), 36.8 (CH), 34.8 (CH_2), 25.0 (CH_2), 14.8 (CH_3), 10.8 (CH_3), 9.1 (CH_3), 7.7 (CH_3) ppm. **Optical rotation**: $[\alpha]^{20}_D$ = –3.3 (*c* 0.3, ethanol).

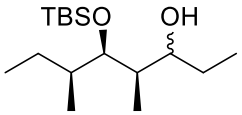
4.2.25 (2*R*,3*R*,4*S*)-2,3-Epoxy-2,4-dimethylhexan-1-ol (150b)



This compound was prepared via the same procedure as described above for **150a**, only (–)-diethyl D-tartrate was used instead of (+)-diethyl L-tartrate. Starting from **149** (2.0 g, 16.6 mmol), the final purification by flash column chromatography on silica gel yielded **150b** (2.11 g, 14.6 mmol, 88%, *dr* > 95:5 by GC/MS analysis) as colourless oil.

TLC (hexane/ethyl acetate = 1:1) R_f = 0.47. **IR** (ATR) $\tilde{\nu}$ = 2960 (m), 2932 (m), 2881 (w), 2859 (w), 1708 (m), 1462 (w), 1383 (w), 1254 (w), 1182 (w), 1057 (m), 1022 (m), 962 (w), 940 (m), 835 (s), 810 (m), 773 (s), 674 (m) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 230 (2.27), 222 (2.10) nm. **MS** (EI, 70 eV): *m/z* (%) = 126 (0.5) $[M-H_2O]^+$, 111 (1), 97 (4), 87 (11), 75 (13), 74 (9), 71 (10), 70 (16), 69 (33), 67 (5), 59 (22), 58 (58), 57 (58), 56 (7), 55 (74), 53 (10), 51 (6), 45 (46), 43 (100), 42 (20), 41 (68), 40 (8), 39 (52), 38 (5). **GC** (BPX-5): *I* = 1106. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 3.68 (dd, $^4J_{H,H}$ = 4.7 Hz, $^3J_{H,H}$ = 12.2 Hz, 1H, CH_2), 3.57 (dd, $^3J_{H,H}$ = 8.2, 12.2 Hz, 1H, CH_2), 2.77 (d, $^3J_{H,H}$ = 8.9 Hz, 1H, CH), 2.05-2.02 (dd, $^4J_{H,H}$ = 4.7 Hz, $^3J_{H,H}$ = 8.1 Hz, 1H, OH), 1.63 (p, $^3J_{H,H}$ = 7.6 Hz, 1H, CH), 1.40-1.31 (m, 2H, CH_2), 1.30 (s, 3H, CH_3), 0.97 (t, $^3J_{H,H}$ = 7.3 Hz, 3H, CH_3), 0.9 (d, $^3J_{H,H}$ = 6.6 Hz, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 65.6 (CH_2), 64.9 (CH), 60.3 (C), 34.1 (CH), 27.9 (CH_2), 15.6 (CH_3), 14.2 (CH_3), 11.3 (CH_3) ppm. **Optical rotation**: $[\alpha]_D^{20}$ = +20.0 (c 0.1, ethanol).

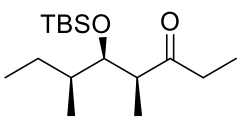
4.2.26 (4*R*,5*R*,6*S*)-5-((*tert*-Butyldimethylsilyl)oxy)-4,6-dimethyloctan-3-ol (152b)



The same two-step procedure as for the preparation of **152a** was used. The starting material **150b** (1.31 g, 9.1 mmol) was converted into the target compound **152b** (0.7 g, 2.43 mmol, 27%) as a mixture of diastereomers (*dr* = 2:1) that was obtained as a colourless oil.

TLC (hexane/ethyl acetate = 10:1) R_f = 0.35. **IR** (ATR) $\tilde{\nu}$ = 3450 (w), 2959 (m), 2932 (m), 2880 (w), 2859 (w), 1463 (m), 1383 (w), 1253 (m), 1047 (m), 1002 (m), 968 (m), 833 (s), 772 (s), 674 (m) cm^{-1}.**UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 242 (2.95) nm. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 3.66 (t, $^4J_{H,H}$ = 3.9 Hz, 1H, CH), 3.54 (m, 1H, CH), 1.79 (s, 1H, OH), 1.67-1.64 (m, 1H, CH), 1.60-1.52 (m, 2H, CH_2), 1.49-1.45 (m, 2H, CH_2), 1.12-1.09 (m, 1H, CH), 0.91 (s, 15H, 5 x CH_3), 0.92-0.87 (m, 6H, 2 x CH_3), 0.08 (d, $^3J_{H,H}$ = 5.0 Hz, 6H, 2 x CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 79.0 (CH), 76.3 (CH), 40.1 (CH), 39.6 (CH), 28.1 (CH_2), 26.1 (3 x CH_3), 25.8 (CH_2), 18.4 (C), 14.8 (CH_3), 12.5 (CH3), 10.5 (CH_3), 8.7 (CH_3), -3.4 (CH_3), -4.2 (CH_3) ppm. Optical rotation: $[\alpha]_D^{20}$ = +2.5 (c 0.1, ethanol).

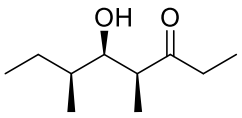
4.2.27 (4*S*,5*R*,6*S*)-5-((*tert*-Butyldimethylsilyl)oxy)-4,6-dimethyloctan-3-one (153b)



The same procedure as for **153a** was used. The starting material **152b** (381 mg, 1.32 mmol) was converted into **153b** (328 mg, 1.14 mmol, 86%) that was obtained as colourless oil.

TLC (hexane/ethyl acetate = 10:1) R_f = 0.55. **IR** (ATR) $\tilde{\nu}$ = 2960 (m), 2932 (m), 2881 (w), 2858 (w), 1712 (m), 1674 (w), 1461 (m), 1381 (w), 1253 (m), 1099 (m), 1051 (m), 1007 (m), 973 (m), 834 (s), 803 (m), 772 (s), 673 (m) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 287 (2.56), 232 (4.29) nm. **MS** (EI, 70 eV): *m/z* (%) = 271 (1) $[M\text{-}CH_3]^+$, 230 (17), 229 (100), 201 (12), 173 (10), 145 (14), 143 (76), 115 (13), 75 (52), 73 (21), 57 (21). **GC** (HP-5MS): *I* = 1591. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 3.85 (dd, $^4J_{H,H}$ = 2.9 Hz, $^3J_{H,H}$ = 6.7 Hz, 1H, CH), 2.73 (p, $^3J_{H,H}$ = 7.0 Hz, 1H, CH), 2.56-2.42 (m, 2H, CH_2), 1.47-1.26 (m, 1H, CH_2), 1.17-1.09 (m, 1H, CH_2), 1.08-1.02 (m, 1H, CH), 0.90 (s, 15H, 5 x CH_3), 0.87 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, CH_3), 0.80 (d, $^3J_{H,H}$ = 6.8 Hz, 3H, CH_3), 0.05 (d, $^3J_{H,H}$ = 6.3 Hz, 6H, 2 x CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 214.0 (C), 76.0 (CH), 49.8 (CH), 39.5 (CH), 35.4 (CH_2), 26.5 (CH_2), 25.8 (3 x CH_3), 18.1 (C), 13.9 (CH_3), 13.6 (CH_3), 11.9 (CH_3), 7.3 (CH_3), -4.2 (CH_3), -4.4 (CH_3) ppm. **Optical rotation**: $[\alpha]_D^{20}$ = +13.2 (c 0.36, ethanol).

4.2.28 (4*S*,5*R*,6*S*)-5-Hydroxy-4,6-dimethyloctan-3-one (137b)



The same procedure as for **137a** was used. The ketone **153b** (162 mg, 0.57 mmol) was converted into **137b** (71 mg, 0.41 mmol, 72%) that was obtained as a colourless oil.

TLC (hexane/ethyl acetate = 5:1) R_f = 0.33. **IR** (ATR) $\tilde{\nu}$ = 3483 (w), 2966 (w), 2936 (w), 2878 (w), 1702 (s), 1459 (m), 1410 (w), 1378 (m), 1237

(w), 1148 (w), 1103 (m), 974 (m), 805 (w), 773 (w), 540 (m) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 224 (3.68), 202 (4.06) nm. **MS** (EI, 70 eV): *m/z* (%) = 154 (6) $[M\text{-}H_2O]^+$, 125 (8), 115 (22), 98 (3), 97 (20), 87 (11), 86 (56), 85 (4), 83 (3), 71 (5), 70 (4), 69 (22), 58 (9), 57 (100), 56 (4), 55 (7), 45 (11), 43 (6), 41 (12). **GC** (HP-5MS): *I* = 1242. **^{1}H NMR** (500 MHz, C_6D_6): δ = 3.58 (dd, $^4J_{H,H}$ = 4.7 Hz, $^3J_{H,H}$ = 6.5 Hz, 1H, CH), 2.40 (dq, $^4J_{H,H}$ = 4.7 Hz, $^3J_{H,H}$ = 7.1 Hz, 1H, CH), 2.24 (s, 1H, OH), 2.07-1.92 (m, 2H, CH_2), 0.97 (dd, $^4J_{H,H}$ = 4.2 Hz, $^3J_{H,H}$ = 6.9 Hz, 6H, 2 x CH_3), 0.91 (t, $^3J_{H,H}$ = 7.2 Hz, 3H, CH_3), 0.8 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, CH_3) ppm. **^{13}C NMR** (125 MHz, C_6D_6): δ = 214.5 (C), 74.7 (CH), 48.2 (CH), 37.5 (CH), 34.9 (CH_2), 26.1 (CH_2), 14.5 (CH_3), 11.4 (CH_3), 11.1 (CH_3), 7.8 (CH_3) ppm. **Optical rotation**: $[\alpha]^{20}_D$ = +1.8 (*c* 0.7, Ethanol).

4.2.29 Epimerisation of (4*R*,5*S*,6*S*)-137a and (4*S*,5*R*,6*S*)-137b

To a solution of the ketone (4*R*,5*S*,6*S*)-**137a** or (4*S*,5*R*,6*S*)-**137b** (10 mg, 0.058 mmol) in methanol (0.6 mL), potassium carbonate (17 mg, 0.12 mmol) was added and the mixture was stirred at room temperature overnight. Distilled water (10 mL) was added and the aqueous phase was extracted extracted three times with ethyl acetate. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. A small sample of the epimerisation product was added to the mixture of eight stereoisomers of **137** in a ca. 1:5 ratio, followed by GC-MS analysis.

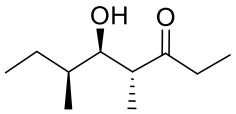
4.2.30 Chromatographic separation of stereoisomers of 137

A portion of the mixture of stereoisomers (100 mg) was subjected to purification. The racemate of **137b** was separated from the other six stereoisomers by column chromatography on silica gel, yielding (*rac*)-**137b** (30 mg). Subsequently, a sample of (*rac*)-**137b** (6.0 mg) was subjected to separation of the enantiomers by HPLC on a chiral

stationary phase (DAICEL Chiralpak IA) to give (4*S*,5*R*,6*S*)-**137b** (3.0 mg) and (4*R*,5*S*,6*R*)-**137b** (3.0 mg).

The mixture of stereoisomers **137a**, **137c** and **137d** (70 mg) was separated by reversed phase HPLC using a KNAUER Europher II 100-5 C18 column to yield (*rac*)-**137d** (12 mg), (*rac*)-**137c** (15 mg) and (*rac*)-**137a** (20 mg). Separation of the racemates of **137c** and **137d** on a chiral stationary phase (DAICEL Chiralpak IA) finally gave pure (4*S*,5*S*,6*S*)-**137c** (7 mg) and (4*R*,5*R*,6*R*)-**137c** (7 mg), and (4*R*,5*R*,6*S*)-**137d** (6 mg) and (4*S*,5*S*,6*R*)-**137d** (6 mg). A separation of the enantiomers of **137a** via HPLC on a chiral column was not successful.

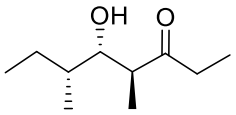
(4*R*,5*R*,6*S*)-5-Hydroxy-4,6-dimethyloctan-3-one (137d)



IR (ATR) $\tilde{\nu}$ = 3479 (w), 2963 (w), 2934 (w), 2877 (w), 1739 (w), 1702 (s), 1458 (m), 1409 (w), 1376 (m), 1299 (w), 1259 (w), 1233 (w), 1136 (w), 1106 (w), 1085 (w), 1021 (m), 972 (m), 956 (m), 867 (m), 799 (m), 736 (m), 702 (w), 516 (w), 446 (w), 409 (w) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 231 (2.99) nm. **MS** (EI, 70 eV): *m/z* (%) = 154 (4) $[M-H_2O]^+$, 125 (9), 116 (4), 115 (57), 113 (5), 98 (6), 97 (24), 87 (9), 86 (46), 85 (4), 83 (6), 71 (4), 70 (8), 69 (35), 58 (8), 57 (100), 56 (5), 55 (8), 45 (7), 43 (5), 41 (11). **GC** (HP-5MS): *I* = 1281.**^{1}H NMR** (500 MHz, C_6D_6): δ = 3.63 (dd, $^4J_{H,H}$ = 3.7 Hz, $^3J_{H,H}$ = 7.5 Hz, 1H, CH), 2.47 (dq, $^3J_{H,H}$ = 7.2, 8.4 Hz, 1H, CH), 2.12 (dd, $^4J_{H,H}$ = 2.4 Hz, $^3J_{H,H}$ = 7.2 Hz, 2H, CH_2), 1.4-1.23 (m, 5H, CH_2, CH_3), 1.22-1.13 (m, 1H, CH), 0.97 (t, $^3J_{H,H}$ = 7.2 Hz, 3H, CH_3), 0.83 (dd, $^4J_{H,H}$ = 3.0 Hz,$^3J_{H,H}$ = 6.9 Hz, 3H, CH_3), 0.81 (s, 1H, OH), 0.76 (d, $^3J_{H,H}$ = 7.2 Hz, 3H, CH_3) ppm. **^{13}C NMR** (125 MHz, C_6D_6): δ = 215.0 (C), 75.8 (CH), 48.8 (CH), 37.0 (CH), 36.1 (CH_2), 27.1 (CH_2), 14.0 (CH_3), 12.5

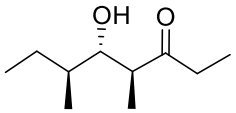
(CH_3), 12.0 (CH_3), 7.8 (CH_3) ppm. **Optical rotation**: $[\alpha]^{20}_D$ = - 2.4 (*c* 0.3, ethanol).

(4*S*,5*S*,6*R*)-5-Hydroxy-4,6-dimethyloctan-3-one (137d)



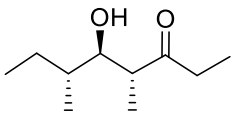
Analytical data were identical to those of (4*S*,5*R*,6*S*)-**137d**. Optical rotation: $[\alpha]_D^{20}$ = +1.3 (c 0.3, ethanol).

(4*S*,5*S*,6*S*)-5-Hydroxy-4,6-dimethyloctan-3-one (137c)



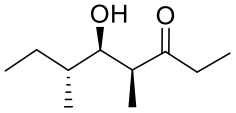
IR (ATR) $\tilde{\nu}$ = 3459 (w), 2962 (w), 2935 (w), 2877 (w), 1710 (m), 1458 (m), 1411 (w), 1377 (m), 1258 (s), 1082 (m), 1010 (s), 864 (m), 790 (s), 734 (w), 700 (w), 661 (w), 407 (w) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 229 (3.39) nm. **MS** (EI, 70 eV): *m/z* (%) = 154 (4) $[M-H_2O]^+$, 125 (9), 116 (4), 115 (62), 114 (5), 98 (5), 97 (22), 87 (8), 86 (34), 85 (4), 83 (5), 71 (4), 70 (6), 69 (31), 58 (8), 57 (100), 56 (4), 55 (8), 45 (6), 43 (4), 41 (10). **GC** (HP-5MS): *I* = 1277. **^{1}H NMR** (500 MHz, C_6D_6): δ = 3.35 (q, $^3J_{H,H}$ = 5.6 Hz, 1H, CH), 2.48 (dq, $^3J_{H,H}$ = 6.3, 7.2 Hz, 1H, CH), 2.16-2.0 (m, 2H, CH_2), 1.65-1.43 (m, 5H, CH_2, CH_3), 1.25-1.15 (m, 1H, CH), 0.93 (t, $^3J_{H,H}$ = 7.2 Hz, 3H, CH_3), 0.86 (dd, $^4J_{H,H}$ = 2.4 Hz, $^3J_{H,H}$ = 7.2 Hz, 3H, CH_3), 0.83 (s, 1H, OH), 0.77 (d, $^3J_{H,H}$ = 6.8 Hz, 3H, CH_3) ppm. **^{13}C NMR** (125 MHz, C_6D_6): δ = 215.7 (C), 78.5 (CH), 47.7 (CH), 38.1 (CH), 36.2 (CH_2), 23.6 (CH_2), 16.3 (CH_3), 14.8 (CH_3), 11.8 (CH_3), 7.7 (CH_3) ppm. **Optical rotation**: $[\alpha]^{20}_D$ = +0.9 (*c* 0.2, ethanol).

(4*R*,5*R*,6*R*)-5-Hydroxy-4,6-dimethyloctan-3-one (137c)



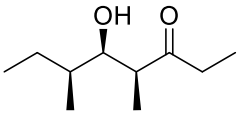
Analytical data were identical to those of (4*R*,5*S*,6*S*)-**137c**. **Optical rotation**: $[\alpha]_D^{20}$= –1.3 (c 0.3, ethanol).

(4*S*,5*R*,6*R*)-5-Hydroxy-4,6-dimethyloctan-3-one (137a)



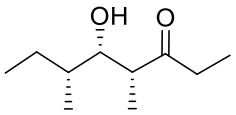
Analytical data of (*rac*)-**137a** were identical to those of (4*R*,5*S*,6*S*)-**137a** obtained by enantioselective synthesis.

(4*S*,5*R*,6*S*)-5-Hydroxy-4,6-dimethyloctan-3-one (137b)



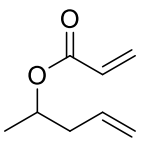
Analytical data were identical to those of (4*S*,5*R*,6*S*)-**137b** obtained by enantioselective synthesis.

(4*R*,5*S*,6*R*)-5-Hydroxy-4,6-dimethyloctan-3-one (137b)



Analytical data were identical to those of (4*S*,5*R*,6*S*)-**137b**. **Optical rotation**: $[\alpha]_D^{20}$ = –1.7 (c 0.7, ethanol).

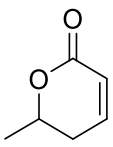
4.2.31 Pent-4-en-2-yl acrylate (175)



To a solution of pent-4-en-2-ol (345 mg, 4.0 mmol) and triethylamine (559 mg, 5.52 mmol) in anhydrous dichloromethane (13.3 mL), acryloyl chloride (362 mg, 4.0 mmol) was added dropwise at 0 °C and stirred at this temperature for 1 hour. Then, it was warmed to room temperature and stirred overnight. The reaction mixture was quenched with 1N HCl and extracted with dichloromethane. The collected organic phases were washed with saturated aqueous $NaHCO_3$ and birne, dried over $MgSO_4$ and concentrated in vacuo to afford **175** as pale yellow oil (367 mg, 2.62 mmol, 66%).

TLC (hexane/ethyl acetate = 40:1) R_f = 0.2. **IR** (ATR) $\tilde{\nu}$ = 3079 (w), 2978 (w), 2930 (w), 2854 (w), 1713 (s), 2637 (w), 1506 (w), 1451 (w), 1406 (w), 1381 (w), 1251 (m), 1192 (m), 1121 (m), 1051 (m), 991 (m), 920 (m), 801 (m), 587 (w) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 251 (4.25), 226 (4.22), 222 (4.17) nm. **MS** (EI, 70 eV): *m/z* (%) = 140 (0.2) $[M]^+$, 100 (1), 99 (24), 96 (2), 69 (4), 68 (8), 67 (6), 56 (4), 55 (100), 53 (5), 45 (3), 43 (7), 41 (20), 39 (18). **GC** (BPX-5): *I* = 922. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = (dd, $^4J_{H,H}$ = 1.5 Hz, $^3J_{H,H}$ = 17.3 Hz, 1H, CH_2), 6.10 (dd, $^3J_{H,H}$ = 10.4, 17.0 Hz, 1H, CH), 5.80 (dd, $^4J_{H,H}$ = 1.5 Hz, $^3J_{H,H}$ = 10.4 Hz, 1H, CH_2), 5.81-5.72 (m, 1H, CH), 5.04 (d, $^3J_{H,H}$ = 6.3 Hz, 1H, CH_2), 5.01 (d, $^3J_{H,H}$ = 6.3 Hz, 1H, CH_2), 5.11-5.06 (m, 1H, CH), 2.39-2.28 (m, 2H, CH_2), 1.28 (d, $^3J_{H,H}$ = 6.3 Hz, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 167 (C), 133.6 (CH), 130.3 (CH_2), 128.9 (CH), 117.7 (CH_2), 70.3 (CH), 40.2 (CH_2), 19.4 (CH_3) ppm.

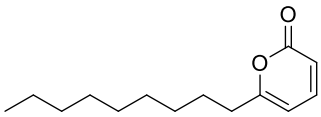
4.2.32 6-Methyl-5,6-dihydro-*2H*-pyran-2-one (169)



To a solution of pent-4-en-2-ylacrylate (290 mg, 2.07 mmol) in anhydrous dichloromethane (41.4 mL), Hoveyda-Grubbs II catalyst (13 mg, 0.021 mmol) was added and stirred under reflux for 4 hours. Then, it was cooled to room temperature and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel to achieve **169** as pale yellow oil (60 mg, 0.54 mmol, 26%).

TLC (hexane/ethyl acetate = 1:1) R_f = 0.2. **IR** (ATR) $\tilde{\nu}$ = 2981 (w), 2937 (w), 2906 (w), 1715 (s), 1389 (m), 1245 (s), 1167 (w), 1107 (m), 1051 (s), 995 (w), 925 (m), 886 (w), 848 (m), 813 (s), 700 (w), 662 (m) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 228 (4.08) nm. **MS** (EI, 70 eV): *m/z* (%) = 112 (3) $[M]^+$, 97 (13), 69 (19), 68 (100), 53 (5), 43 (16), 42 (15), 41 (20), 40 (32), 39 (45), 38 (9), 38 (4). **GC** (HP-5): *I* = 1074. (BPX-5): *I* = 1100. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 6.84-6.79 (m, 1H, CH), 5.97-5.94 (m, 1H, CH), 4.53-4.49 (m, 1H, CH), 2.31-2.24 (m, 2H, CH_2), 1.38 (d, $^3J_{H,H}$ = 6.3 Hz, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 164.5 (C), 144.9 (CH), 121.2 (CH), 74.3 (CH), 30.9 (CH_2), 20.7 (CH_3) ppm. **HRMS-ESI:** calcd. for $C_6H_8O_2$ 112.0524; found 112.0524 $[M]^+$.

4.2.33 6-Nonyl-2*H*-pyran-2-one (171)

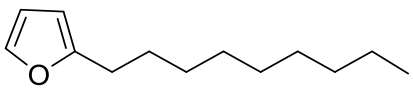


Propiolic acid (49 mg, 0.7 mmol) was added to a solution of 1-Undecyne (106 mg, 0.7 mmol) and chloro(triphenylphosphine)gold (I) catalyst (4 mg, 0.01 mmol) in anhydrous dichloromethane (4 mL) in a sealed tube. Then, silver trifluoromethanesulfonate (2 mg, 0.01 mmol) was added and the tube was closed instantly. After that, it was heated to 50 °C for 15 hours. The solvent was evaporated and the residue was purified over column

chromatography on silica gel to obtain 6-nonyl-2*H*-pyran-2-one **171** (46 mg, 0.12 mmol, 30%) as colourless oil.

TLC (hexane/ethyl acetate = 15:1) R_f = 0.12. **IR** (ATR) $\tilde{\nu}$ = 2923 (s), 2853 (m), 1726 (s), 1633 (m), 1557 (s), 1464 (m), 1397 (w), 1377 (w), 1354 (w), 1266 (w), 1172 (w), 1085 (m), 979 (w), 892 (w), 852 (w), 796 (s), 722 (m), 548 (w), 491 (w) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 300 (4.65), 217 (4.39) nm. **MS** (EI, 70 eV): *m/z* (%) = 222 (14) $[M]^+$, 137 (20), 123 (37), 110 (47), 96 (13), 95 (100), 94 (17), 82 (49), 81 (45), 69 (11), 67 (16), 55 (23), 53 (18), 43 (32), 41 (52), 39 (67). **GC** (HP-5): *I* = 1889. (BPX-5): *I* = 1905. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 7.21-7.17 (m, 1H, CH), 6.09-6.07 (dd, $^4J_{H,H}$ = 0.5 Hz, $^3J_{H,H}$ = 9.3 Hz, 1H, CH), 5.91-5.89 (dd, $^4J_{H,H}$ = 0.8 Hz, $^3J_{H,H}$ = 6.6 Hz, 1H, CH), 2.40 (t, $^3J_{H,H}$ = 7.6 Hz, 2H, CH_2), 1.59 (dt, $^3J_{H,H}$ = 7.5, 15 Hz, 2H, CH_2), 1.28-1.19 (m, 12H, 6 x CH_2), 0.81 (t, $^3J_{H,H}$ = 6.9 Hz, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 166.8 (C), 162.9 (C), 143.7 (CH), 113.0 (CH), 102.6 (CH), 33.8 (CH_2), 31.8 (CH_2), 29.4 (CH_2), 29.2 (2x CH_2), 28.9 (CH_2), 26.9 (CH_2), 22.6 (CH_2), 14.1 (CH_3) ppm. **HRMS-ESI:** calcd. for $C_{14}H_{22}O_2$ 222.1620; found 222.1620 $[M]^+$.

4.2.34 2-Nonylfuran (180)



To a solution of furan (500 mg, 7.3 mmol) in anhydrous THF (25 mL), a solution of *n*-BuLi (1.6 M in hexane, 4.6 mL, 7.30 mmol) was added dropwise at -78 °C and stirred for 30 minutes. Then, a solution of 1-bromo nonane (1.54 g, 7.03 mmol) in dry THF (2 mL) was added. It was stirred at room temperature for another 3 hours. Distilled water (4 mL) was added and the reaction mixture was extracted with diethyl ether. The

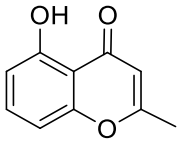
collected organic layers were dried over $MgSO_4$, concentrated in vacuo and purified by flash column chromatography on silica gel to yield 2-nonylfuran **180** (757 mg, 3.90 mmol, 53%) as colourless oil.

TLC (hexane) R_f = 0.51. **IR** (ATR) $\tilde{\nu}$ = 2924 (m), 2854 (m), 1597 (w), 1507 (w), 1464 (w), 1379 (w), 1146 (w), 1077 (w), 1006 (w), 924 (w), 885 (w), 794 (w), 723 (s), 599 (w) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 277 (2.85), 229 (4.56) nm. **MS** (EI, 70 eV): *m/z* (%) = 194 (13) $[M]^+$, 151 (6), 137 (6), 123 (8), 96 (7), 95 (7), 94 (8), 82 (35), 81 (100), 67 (6), 55 (5), 53 (12), 43 (8), 41 (12), 39 (5). **GC** (HP-5): *I* = 1397. (BPX-5): *I* = 1399. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 7,29 (dd, $^3J_{H,H}$ = 0.9 Hz, 1.9 Hz, 1H, CH), 6.27 (dd, $^3J_{H,H}$ = 1.9, 3.1 Hz, 1H, CH), 5.97-5.96 (m, 1H, CH), 2.61 (t, $^3J_{H,H}$ = 7.6 Hz, 2H, CH_2), 1.66-1.59 (m, 2H, CH_2), 1.35-1.24 (m, 12H, 6 x CH_2), 0.89-0.86 (m, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 156.7 (CH), 140.6 (C), 110.0 (CH), 104.5 (CH), 31.9 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 28.1 (CH_2), 28.0 (CH_2), 22.7 (CH_2), 14.1 (CH_3) ppm. **HRMS-ESI:** calcd. for $C_{13}H_{22}O$ 194.1671; found 194.1667 $[M]^+$.

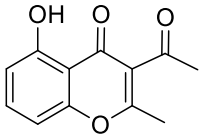
4.2.35 5-Hydroxy-2-methyl-4*H*-chromen-4-one (214) and 3-acetyl-5-hydroxy-2-methyl-4*H*-chromen-4-one (220)

To a solution of 1-(2,6-dihydroxyphenyl)ethanone (304 mg, 2.0 mmol) in acetone (10 mL), potassium carbonate (1.38 g, 10.0 mmol) was added and stirred at room temperature for 15 minutes. Then, acetyl chloride (157 mg, 2.0 mmol) was added and stirred under reflux for 24 hours. It was cooled to room temperature, washed with distilled water, extracted with diethyl ether. The collected organic phases were dried over $MgSO_4$, concentrated in vacuo and purified by flash column chromatography over silica gel to afford 5-hydroxy-2-methyl-4*H*-chromen-4-one **214** (37 mg, 0.21 mmol, 11%) as yellow solid and side product 3-acetyl-5-hydroxy-2-

methyl-4*H*-chromen-4-one **220** (154 mg, 0.71 mmol, 35%) as yellow solid.



TLC (hexane/ethyl acetate/toluene = 3:1:1) R_f = 0.36. **IR** (ATR) $\tilde{\nu}$ = 3075 (w), 2970 (w), 2930 (w), 2850 (w), 2781 (w), 1664 (s), 1622 (s), 1596 (s), 1465 (m), 1404 (s), 1377 (s), 1305 (s), 1250 (s), 1228 (s), 1183 (m), 1158 (m), 1110 (m), 1061 (m), 1002 (m), 953 (s), 867 (m), 838 (m), 799 (s), 745 (s), 741 (m), 681 (m), 617 (m), 587 (s) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 325 (4.64), 253 (5.08), 230 (5.30) nm. **MS** (EI, 70 eV): *m/z* (%) = 177 (12) $[M+H]^+$, 176 (100), 148 (32), 147 (26), 136 (22), 108 (37), 91 (10), 39 (12). **GC** (HP-5): *I* = 1640. (BPX-5): *I* = 1592. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 12.54 (s, 1H, OH), 7.48 (t, $^3J_{H,H}$ = 8.3 Hz, 1H, CH), 6.84 (dd, $^4J_{H,H}$ = 0.9 Hz, $^3J_{H,H}$ = 8.5 Hz, 1H, CH), 6.76 (dd, $^4J_{H,H}$ = 0.9 Hz, $^3J_{H,H}$ = 8.3 Hz, 1H, CH), 6.09 (s, 1H, CH), 2.38 (s, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 183.6 (C), 167.7 (C), 160.9 (C), 156.9 (C), 135.2 (CH), 111.3 (CH), 110.6 (C), 109.3 (CH), 106.9 (CH), 20.8 (CH_3) ppm. **HRMS-ESI:** calcd. for $C_{10}H_8O_3$ 176.0473; found 176.0472 $[M]^+$.

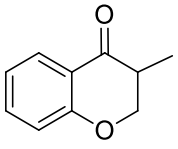


TLC (hexane/ethyl acetate/toluene = 3:1:1) R_f = 0.33. **IR** (ATR) $\tilde{\nu}$ = 3073 (w), 2965 (w), 2925 (w), 2851 (w), 1692 (s), 1642 (s), 1601 (s), 1503 (m), 1469 (s), 1409 (s), 1376 (m), 1348 (m), 1296 (s), 1213 (s), 1164 (m), 1129 (m), 1078 (m), 1057 (m), 1036 (m), 994 (m), 953 (m), 881 (m), 861 (m), 812 (s), 756 (s), 707 (s), 650 (m), 634 (m), 593 (m), 531 (m) cm^{-1}.

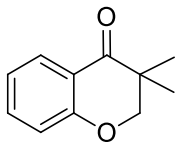
UV-Vis (CH_2Cl_2) λ_{max} (log ε) 328 (4.65), 241 (5.17), 228 (5.19) nm. **MS** (EI, 70 eV): *m/z* (%) = 219 (11) $[M+H]^+$, 218 (88), 204 (13), 203 (100), 137 (53), 136 (17), 108 (21), 67 (26), 43 (23), 39 (13). **GC** (HP-5): *I* = 1800. (BPX-5): *I* = 1839. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 12.42 (s, 1H, OH), 7.52 (t, $^3J_{H,H}$ = 8.4 Hz, 1H, CH), 6.86 (dd, $^4J_{H,H}$ = 0.9 Hz, $^3J_{H,H}$ = 8.4 Hz, 1H, CH), 6.80 (d, $^4J_{H,H}$ = 0.9 Hz, $^3J_{H,H}$ = 8.3 Hz, 1H, CH), 6.09 (s, 1H, CH), 2.62 (s, 3H, CH_3), 2.52 (s, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 199.3 (C), 181.5 (C), 170.4 (C), 161.1 (C), 155.7 (C), 135.9 (CH), 122.3 (C), 112.2 (CH), 110.5 (CH), 106.9 (CH), 32.4 (CH_3), 20.2 (CH_3) ppm. **HRMS-ESI:** calcd. for $C_{12}H_{10}O_4$ 218.0579; found 218.0579 $[M]^+$.

4.2.36 3-Methylchroman-4-one (215)

To a solution of diisopropylamine (202 mg, 2.0 mmol) in anhydrous THF (5.0 mL), *n*-BuLi (1.25 mL, 2.0 mmol, 1.6 M in Hexane) was added at 0 °C and stirred at this temperature for 1 hour, then cooled to - 78 °C. 4-Chromanone (296 mg, 2.0 mmol) in anhydrous THF (1.0 mL) was put in and maintained this temperature for 1 hour. After that, methyl iodide (284 mg, 2.0 mmol) was added to the reaction mixture and stirred at -78 °C for 1 hour and maintained at 0 °C overnight. It was washed with saturated NH_4Cl aqueous solution, extracted with ethyl acetate, dried over $MgSO_4$, concentrated in vacuo and purified by flash column chromatography on silica gel to provide 3-methylchroman-4-one **215** as colourless oil (100 mg, 0.62 mmol, 31%) and 3,3-dimethylchroman-4-one (81 mg, 0.46 mmol, 23 %) as colourless oil as well.



TLC (cyclohexane/ethyl acetate = 5:1) R_f = 0.4. **IR** (ATR) $\tilde{\nu}$ = 2971 (w), 2932 (w), 2875 (w), 1687 (m), 1604 (m), 1579 (w), 1477 (m), 1467 (m), 1454 (m), 1386 (w), 1386 (m), 1323 (m), 1294 (m), 1247 (m), 1209 (m), 1147 (m), 1128 (w), 1104 (w), 1071 (m), 1037 (m), 1013 (m), 970 (m), 960 (m), 940 (w), 895 (w), 825 (m), 772 (m), 753 (s), 691 (m), 676 (w), 650 (w), 576 (w), 533 (m), 518 (w), 436 (w) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 320 (3.70), 251 (4.09), 214 (4.27) nm. **MS** (EI, 70 eV): *m/z* (%) = 163 (10) $[M+H]^+$, 162 (94), 121 (18), 120 (100), 93 (7), 92 (90), 77 (4), 76 (4), 65 (7), 64 (18), 63 (16), 50 (4), 39 (9). **GC** (HP-5MS): *I* = 1367. **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 7.89 (dd, $^4J_{H,H}$ = 1.8 Hz, $^3J_{H,H}$ = 7.8 Hz, 1H, CH), 7.45 (ddd, $^4J_{H,H}$ = 1.8 Hz, $^3J_{H,H}$ = 8.3, 7.2 Hz, 1H, CH), 7.00 (ddd, $^4J_{H,H}$ = 1.1 Hz, $^3J_{H,H}$ = 8.0, 7.2 Hz, 1H, CH), 6.95 (dd, $^4J_{H,H}$ = 1.0 Hz, $^3J_{H,H}$ = 8.4 Hz, 1H, CH_2), 4.49 (dd, $^3J_{H,H}$ = 11.3, 5.1 Hz, 1H, CH_2), 4.14 (t, $^3J_{H,H}$ = 11.1 Hz, 1H), 2.85 (m, 1H, CH), 1.21 (d, $^3J_{H,H}$ = 7.0 Hz, 3H, CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 194.9 (C), 161.8 (C), 136.9 (CH), 127.5 (CH), 121.5 (CH), 120.7 (C), 117.8 (CH), 72.3 (CH_2), 40.8 (CH), 10.8 (CH_3) ppm. **HRMS-ESI:** calcd. for $C_{10}H_{10}O_2$ 162.0681; found 162.0681 $[M]^+$.



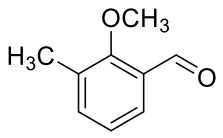
TLC (cyclohexane/ethyl acetate = 5:1) R_f = 0.49. **IR** (ATR) $\tilde{\nu}$ = 3069 (w), 2966 (w), 2929 (w), 2869 (w), 1687 (m), 1605 (m), 1580 (w), 1476 (m), 1464 (m), 1453 (m), 1392 (w), 1362 (w), 1314 (m), 1277 (m), 1256 (w), 1240 (w), 1209 (m), 1182 (w), 1146 (m), 1105 (m), 1035 (m), 992 (w), 950 (m), 931 (w), 865 (w), 818 (w), 789 (w), 757 (m), 699 (m), 658 (w), 574 (w), 525 (m), 473 (w), 451 (w), 431 (w) cm^{-1}. **UV-Vis** (CH_2Cl_2) λ_{max} (log ε) 318 (2.68), 251 (3.08), 214 (3.48) nm. **MS** (EI, 70 eV): *m/z* (%) =

177 (4) $[M+H]^+$, 176 (37), 161 (7), 121 (19), 120 (100), 92 (33), 65 (4), 64 (6), 63 (5), 39 (4). **GC** (HP-5MS): *I* = 1365. **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 7.90 (dd, $^4J_{H,H}$ = 1.8 Hz, $^3J_{H,H}$ = 7.8 Hz, 1H, CH), 7.46 (ddd, $^4J_{H,H}$ = 1.8 Hz, $^3J_{H,H}$ = 8.4, 7.1 Hz, 1H, CH), 7.01 (ddd, $^4J_{H,H}$ = 1.1 Hz, $^3J_{H,H}$ = 8.1, 7.2 Hz, 1H, CH), 6.95 (dd, $^4J_{H,H}$ = 1.0 Hz, $^3J_{H,H}$ = 8.4 Hz, 1H, CH), 4.14 (s, 2H, CH_2), 1.20 (s, 6H, 2 x CH_3) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): δ = 197.4 (C), 161.3 (C), 135.7 (CH), 127.9 (CH), 121.6 (CH), 119.7 (C), 117.8 (CH), 76.8 (CH_2), 41.8 (C), 20.6 (2 x CH_3) ppm. **HRMS-ESI:** calcd. for $C_{11}H_{12}O_2$ 176.0837; found 176.0838 $[M]^+$.

4.2.37 Preparation of methoxy-methyl-benzaldehydes

To a solution of the hydroxy-methyl-benzaldehyde (34 mg, 0.25 mmol) in anhydrous DMF (6.0 mL), K_2CO_3 (35 mg, 0.25 mmol) was added and the mixture was stirred at room temperature for 30 minutes. Then, methyl iodide (30 μL, 68 mg, 0.5 mmol) was added and the reaction was stirred at room temperature overnight. The reaction was quenched by the addition of distilled water, and the aqueous phase was extracted three times with ethyl acetate. The combined organic phases were dried with $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel.

2-Methoxy-3-methylbenzaldehyde

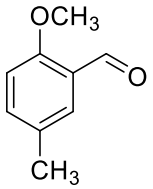


Yield: 26 mg (0.17 mmol, 69%), colourless oil.

TLC (cyclohexane/ethyl acetate = 10:1) R_f = 0.18. (UV) **MS** (EI, 70 eV) *m*/*z* (%) 150 (100) $[M]^+$, 149 (31), 135 (29), 134 (10), 133 (49), 132 (52), 121 (10), 119 (12), 118 (10), 105 (26), 104 (18), 91 (47), 90 (26), 89 (18),

78 (13), 77 (26). **GC** (HP-5MS): *I* = 1265. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 10.38 (d, $^{4}J_{H,H}$ = 0.8 Hz, 1H, CHO), 7.68 (dd, $^{3}J_{H,H}$ = 7.7 Hz, $^{4}J_{H,H}$ = 1.7 Hz, 1H, CH), 7.51-7.40 (m, 1H, CH), 7.13 (t, $^{3}J_{H,H}$ = 7.6 Hz, 1H, CH), 3.88 (s, 3H, OCH_3), 2.34 (s, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 190.4 (CHO), 161.9 (C), 137.7 (CH), 132.4 (C), 129.3 (C), 126.6 (CH), 124.5 (CH), 63.2 (OCH_3), 15.6 (CH_3) ppm.

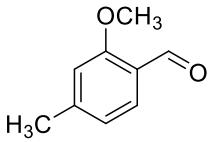
2-Methoxy-5-methylbenzaldehyde



Yield: 33 mg (0.22 mmol, 89%), colourless oil.

TLC (cyclohexane/ethyl acetate = 10:1) R_f = 0.15. (UV) **MS** (EI, 70 eV) *m*/*z* (%) 150 (100) $[M]^+$, 149 (38), 135 (14), 133 (26), 132 (20), 121 (10), 118 (10), 105 (18), 104 (17), 91 (33), 90 (13), 89 (13), 77 (19). **GC** (HP-5MS): *I* = 1352. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 10.43 (d, $^{4}J_{H,H}$ = 0.7 Hz, 1H, CHO), 7.61 (d, $^{4}J_{H,H}$ = 2.4 Hz, 1H, CH), 7.34 (dd, $^{3}J_{H,H}$ = 8.5 Hz, $^{4}J_{H,H}$ = 2.4 Hz, 1H, CH), 6.88 (t, $^{3}J_{H,H}$ = 8.5 Hz, 1H, CH), 3.89 (s, 3H, OCH_3), 2.30 (s, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 190.1 (CHO), 160.1 (C), 136.7 (CH), 130.1 (C), 128.7 (CH), 124.6 (C), 111.7 (CH), 55.8 (OCH_3), 20.3 (CH_3) ppm.

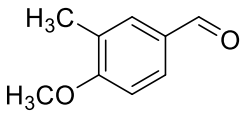
2-Methoxy-4-methylbenzaldehyde (198)



Yield: 35 mg (0.23 mmol, 91%), white solid.

TLC (cyclohexane/ethyl acetate = 10:1) R_f = 0.18. (UV) **MS** (EI, 70 eV) *m*/*z* (%) 150 (100) $[M]^+$, 149 (60), 135 (11), 133 (39), 132 (26), 118 (28), 105 (21), 104 (15), 91 (37), 90 (15), 89 (13), 77 (15). **GC** (HP-5MS): *I* = 1372. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 10.38 (s, 1H, CHO), 7.71 (d, $^3J_{H,H}$ = 7.8 Hz, 1H, CH), 6.84-6.80 (m, 1H, CH), 6.77 (s, 1H, CH), 3.90 (s, 3H, OCH_3), 2.40 (s, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 189.6 (CHO), 162.0 (C), 147.5 (C), 128.7 (CH), 122.8 (C), 121.8 (CH), 112.3 (CH), 55.7 (OCH_3), 22.4 (CH_3) ppm.

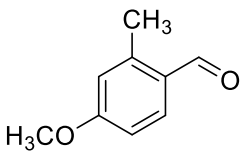
4-Methoxy-3-methylbenzaldehyde



Yield: 32 mg (0.21 mmol, 84%), white solid.

TLC (cyclohexane/ethyl acetate = 10:1) R_f = 0.15. (UV) **MS** (EI, 70 eV) *m*/*z* (%) 150 (69) $[M]^+$, 151 (6), 149 (100), 121 (7), 106 (3), 91 (17), 89 (2), 78 (3), 77 (8), 65 (2). **GC** (HP-5MS): *I* = 1366. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 9.84 (s, 1H, CHO), 7.70 (dd, $^3J_{H,H}$ = 8.4 Hz, $^4J_{H,H}$ = 2.2 Hz, 1H, CH), 7.67 (dd, $^4J_{H,H}$ = 2.2, 1.0 Hz, 1H, CH), 6.91 (d, $^3J_{H,H}$ = 8.4 Hz, 1H, CH), 3.90 (s, 3H, OCH_3), 2.25 (s, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 191.3 (CHO), 163.0 (C), 131.6 (CH), 130.8(CH), 129.6 (C), 127.8 (C), 109.8 (CH), 55.8 (OCH_3), 16.3 (CH_3) ppm.

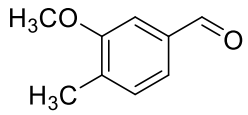
4-Methoxy-2-methylbenzaldehyde



Yield: 32 mg (0.21 mmol, 84%), yellow oil.

TLC (cyclohexane/ethyl acetate = 10:1) R_f = 0.18. (UV) **MS** (EI, 70 eV) *m*/*z* (%) 150 (65) $[M]^+$, 151 (6), 149 (100), 122 (3), 121 (17), 106 (2), 91 (12), 89 (2), 78 (4), 77 (9), 63 (2), 51 (3). **GC** (HP-5MS): *I* = 1368. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 10.38 (s, 1H, CHO), 7.75 (d, $^3J_{H,H}$ = 8.5 Hz, 1H, CH), 6.84 (dd, $^3J_{H,H}$ = 8.5 Hz, $^4J_{H,H}$ = 2.5 Hz 1H, CH), 6.76-6.73 (m, 1H, CH), 3.86 (s, 3H, OCH_3), 2.65 (s, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 191.3 (CHO), 163.8 (C), 143.4 (C), 134.9 (CH), 128.1 (C), 117.1 (CH), 111.6 (CH), 55.6 (OCH_3), 20.0 (CH_3) ppm.

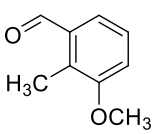
3-Methoxy-4-methylbenzaldehyde



Yield: 35 mmol (0.23 mmol, 91%), white solid.

TLC (cyclohexane/ethyl acetate = 10:1) R_f = 0.18. (UV) **MS** (EI, 70 eV) *m*/*z* (%) 150 (100) $[M]^+$, 151 (9), 149 (89), 135 (4), 122 (4), 121 (22), 106 (4), 91 (25), 89 (3), 79 (3), 78 (5), 77 (13), 65 (4), 51 (3), 44 (3). **GC** (HP-5MS): *I* = 1307. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 9.92 (s, 1H, CHO), 7.36 (dd, $^3J_{H,H}$ = 7.5 Hz, $^4J_{H,H}$ = 1.5 Hz, 1H, CH), 7.33 (d, $^4J_{H,H}$ = 1.4 Hz, 1H, CH), 7.29 (dd, $^3J_{H,H}$ = 7.3 Hz, $^4J_{H,H}$ = 1.0 Hz, 1H, CH), 3.89 (s, 3H, OCH_3), 2.29 (s, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 192.1 (CHO), 158.5 (C), 136.0 (C), 135.0 (C), 131.0 (CH), 124.6 (CH), 108.0 (CH), 55.6 (OCH_3), 17.0 (CH_3) ppm.

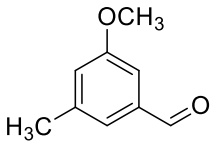
3-Methoxy-2-methylbenzaldehyde



Yield: 27 mg (0.18 mmol, 72%), yellow oil.

TLC (cyclohexane/ethyl acetate = 10:1) R_f = 0.24. (UV) **MS** (EI, 70 eV) *m*/*z* (%) 150 (100) $[M]^+$, 151 (9), 149 (40), 135 (5), 121 (15), 120 (12), 119 (10), 107 (8), 105 (8), 91 (39), 79 (7), 78 (7), 77 (20), 51 (5). **GC** (HP-5MS): *I* = 1335. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 10.64 (d, $^4J_{H,H}$ = 0.6 Hz, 1H, CHO), 7.37 (dd, $^3J_{H,H}$ = 8.4, 7.6 Hz, 1H, CH), 6.83 (d, $^3J_{H,H}$ = 8.4 Hz, 1H, CH), 6.79 (dq, $^3J_{H,H}$ = 7.6 Hz, $^4J_{H,H}$ = 0.8 Hz, 1H, CH), 3.89 (s, 3H, OCH_3), 2.56 (s, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 192.4 (CHO), 163.4 (C), 142.1 (C), 134.6 (CH), 124.2 (CH), 123.5 (C), 109.2 (CH), 55.9 (OCH_3), 21.6 (CH_3) ppm.

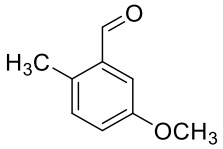
3-Methoxy-5-methylbenzaldehyde



Yield: 28 mg (0.19 mmol, 75%), yellow oil.

TLC (cyclohexane/ethyl acetate = 10:1) R_f = 0.23. (UV) **MS** (EI, 70 eV) *m*/*z* (%) 150 (100) $[M]^+$, 151 (9), 149 (85), 122 (5), 121 (33), 119 (3), 106 (3), 105 (2), 92 (2), 91 (16), 89 (2), 79 (3), 78 (4), 77 (10), 65 (3), 63 (2), 51 (2). **GC** (HP-5MS): *I* = 1313. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 9.95 (s, 1H, CHO), 7.29 (tt, $^4J_{H,H}$ = 1.7, 0.7 Hz, 1H, CH), 7.22 (dd, $^4J_{H,H}$ = 2.6, 1.3 Hz, 1H, CH), 7.01 (ddd, $^4J_{H,H}$ = 2.4, 1.5, 0.8 Hz, 1H, CH), 3.86 (s, 3H, OCH_3), 2.42 (d, $^4J_{H,H}$ = 0.9 Hz, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 192.5 (CHO), 160.3 (C), 140.5 (C), 137.9 (C), 124.5 (CH), 122.3 (CH), 109.6 (CH), 55.6 (OCH_3), 21.3 (CH_3) ppm.

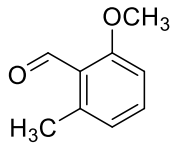
5-Methoxy-2-methylbenzaldehyde



Yield: 21 mg (0.14 mmol, 56%), colourless oil.

TLC (cyclohexane/ethyl acetate = 10:1) R_f = 0.19. (UV) **MS** (EI, 70 eV) *m*/*z* (%) 150 (100) $[M]^+$, 151 (9), 149 (45), 135 (4), 122 (9), 121 (68), 119 (2), 107 (7), 106 (2), 92 (2), 91 (13), 89 (3), 79 (4), 78 (6), 77 (14), 65 (3), 63 (2), 51 (3). **GC** (HP-5MS): *I* = 1323. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 10.27 (s, 1H, CHO), 7.32 (d, $^4J_{H,H}$ = 2.9 Hz, 1H, CH), 7.16 (d, $^3J_{H,H}$ = 8.2 Hz, $^4J_{H,H}$ = 0.9 Hz, 1H, CH), 7.04 (dd, $^3J_{H,H}$ = 8.4 Hz, $^4J_{H,H}$ = 2.9 Hz, 1H, CH), 3.88 (s, 3H, OCH_3), 2.60 (s, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 192.2 (CHO), 158.2 (C), 134.8 (C), 133.1 (C), 132.9 (CH), 120.9 (CH), 114.2 (CH), 55.6 (OCH_3), 18.2 (CH_3) ppm.

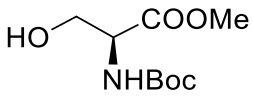
2-Methoxy-6-methylbenzaldehyde



Yield: 27 mg (0.18 mmol, 72%), white solid.

TLC (cyclohexane/ethyl acetate = 10:1) R_f = 0.29. (UV) **MS** (EI, 70 eV) *m*/*z* (%) 150 (100) $[M]^+$, 151 (10), 149 (47), 135 (16), 134 (6), 133 (27), 132 (13), 119 (5), 118 (15), 105 (13), 104 (7), 91 (30), 90 (19), 89 (12), 79 (6), 78 (9), 77 (14). **GC** (HP-5MS): *I* = 1335. **^{1}H NMR** (400 MHz, $CDCl_3$, TMS): δ = 10.64 (d, $^4J_{H,H}$ = 0.6 Hz, 1H, CHO), 7.37 (dd, $^3J_{H,H}$ = 8.4, 7.6 Hz, 1H, CH), 6.83 (d, $^3J_{H,H}$ = 8.4 Hz, 1H, CH), 6.79 (dq, $^3J_{H,H}$ = 7.6 Hz, $^4J_{H,H}$ = 0.8 Hz, 1H, CH), 3.89 (s, 3H, OCH_3), 2.56 (s, 3H, CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$, TMS): δ = 192.4 (CHO), 163.3 (C), 142.1 (C), 134.6 (CH), 124.2 (CH), 123.5 (C), 109.2 (CH), 55.9 (OCH_3), 21.6 (CH_3) ppm.

4.2.38 (*S*)-Methyl 2-((*tert*-butoxycarbonyl)amino)-3-hydroxypropanoate (264)



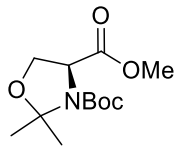
To a solution of L-serine (15.85 g, 0.15 mol) in 1N sodium hydroxide aqueous solution (320 mL), a solution of di-*tert*-butyl dicarbonate (39.2 g, 0.18 mol) in 1,4-dioxane (140 mL) was added at 0 °C and stirred 30 minutes at this temperature. Then, it was warmed to room temperature and stirred for another 3.5 hours. The reaction mixture was cooled in an ice bath, acidified to pH 2-3 by addition of 1N potassium bisulfate. The aqueous phase was extracted with ethyl acetate. The combined organic phases were dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and used for next step without purification.

To a solution of N-Boc-L-serine (0.15 mol) in dimethylformamide (140 mL), potassium carbonate (22.78 g, 0.165 mol) was added at 0 °C and stirred for 10 minutes. Then, methyl iodide (18.68 mL, 42.6 g, 0.3 mol) was added dropwise at 0 °C and stirred another 30 minutes at this temperature. The reaction was allowed to warm to room temperature and stirred overnight.The reaction mixture was filtered and washed with ethyl acetate and distilled water. The collected organic layers were washed with brine, dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to afford **264** (28.87 g. 0.13 mol, 90% over two steps) as pale oil.

TLC (cyclohexane/ethyl acetate = 1:1) R_f =0.38. **UV-Vis** (EtOH) λ_{max} (log ε) 201 (3.13) nm. **IR** (ATR) $\tilde{\nu}$ = 3390 (m), 2977 (w), 1688 (s), 1506 (m), 1456 (m), 1438 (w), 1392 (w), 1366 (m), 1349 (w), 1284 (m), 1209 (m),

1158 (s), 1058 (m), 1029 (m), 921 (w), 872 (w), 851 (w), 810 (w), 779 (w), 759 (w), 528 (w), 461 (w) cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 5.60-5.45 (m, 1H, NH), 4.35 (s, 1H, CH_2), 3.90 (qd, $^{3}J_{H,H}$ = 11.2, 3.8 Hz, 2H, CH, CH_2), 3.76 (s, 3H, CH_3), 2.61 (s, 1H, OH), 1.43 (s, 9H, 3 x CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 171.5(C), 155.9 (C), 80.4 (C), 63.5 (CH_2), 55.8 (CH), 52.7 (CH), 28.4 (3 x CH_3) ppm. **Optical rotation**: $[\alpha]_D^{20}$ = -2.3 (*c* 0.5, ethanol). **HRMS-ESI**: calcd. for $C_9H_{17}NNaO_5^+$ 242.0999; found 242.0999.

4.2.39 (*S*)-3-*tert*-Butyl 4-methyl 2,2-dimethyloxazolidine-3,4-dicarboxylate (265)

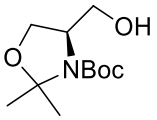


In a 1 L three-necks round flask with reflux condenser, N-Boc-L-serine methyl ester **264** (28.87 g, 0.13 mol), 2,2-dimethoxypropane (34.32 g, 0.33 mol) and *p*-toluenesulfonic acid monohydrate (380 mg, 2.0 mmol) in benzene (438 mL, 0.3 mol/L) were put in and heated at 110 °C for 4 hours. The reaction was monitored by TLC. Then, it was cooled down, quenched with saturated $NaHCO_3$ aqueous solution, extracted with diethyl ether. The collected organic phases were washed with birne, dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to yield **265** (30.3 g, 0.117 mol, 89%, *dr* 62:38) as pale oil.

TLC (cyclohexane/ethyl acetate = 5:1) R_f =0.3. **IR** (ATR) $\tilde{\nu}$ = 2978 (w), 1757 (m), 1699 (s), 1479 (w), 1456 (w), 1437 (w), 1377 (s), 1364 (s), 1250 (m), 1202 (m), 1163 (s), 1091 (s), 1066 (m), 1053 (m), 1000 (w), 945 (w), 846 (m), 807 (w), 769 (m), 659 (w), 639 (w), 553 (w), 515 (w),

463 (w), 419 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 201 (3.17) nm. **MS** (EI, 70 eV): *m/z* (%) = 244 (18) $[M\text{-}CH_3]^+$, 188 (8), 186 (13), 146 (4), 144 (100), 128 (18), 99 (22), 86 (6), 84 (17), 83 (5), 59 (6), 58 (7), 57 (91), 56 (10), 55 (3), 43 (6), 42 (4), 41 (16), 39 (3). **GC** (HP-5MS): *I* = 1443. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = **A**: 4.47 (dd, $^3J_{H,H}$ = 6.7, 2.7 Hz, 1H, CH), 4.12 (dt, $^3J_{H,H}$ = 6.6 Hz, $^2J_{H,H}$ = 9.2 Hz, 2H, CH_2), 3.74 (s, 3H, CH_3), 1.65 (s, 3H, CH_3), 1.62 (s, 3H, CH_3), 1.39 (s, 9H, 3 x CH_3) ppm.; **B**: 4.36 (dd, $^3J_{H,H}$ = 7.0, 3.1 Hz, 1H, CH), 4.07-3.96 (m, 2H, CH_2), 3.74 (s, 3H, CH_3), 1.62 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 1.48 (s, 9H, 3 x CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = **A**: 171.8 (C), 151.3 (C), 95.2 (C), 80.4 (C), 66.4 (CH_2), 59.4 (CH), 52.4 (CH_3), 28.4 (3 x CH_3), 25.3 (CH_3), 24.5 (CH_3) ppm.; **B**: 171.39 (C), 152.2 (C), 94.5 (C), 81.0 (C), 66.1 (CH_2), 59.3 (CH), 52.5 (CH3), 28.5 (3 x CH_3), 26.1 (CH_3), 25.1 (CH_3) ppm. **Optical rotation**: $[\alpha]_D^{20}$ = -4.3 (*c* 0.5, ethanol). **HRMS-ESI**: calcd. for $C_{11}H_{18}NO_5^+$ 244.1185; found 244.1186.

4.2.40 (*R*)-*tert*-Butyl 4-(hydroxymethyl)-2,2-dimethyloxazolidine-3-carboxylate (266)

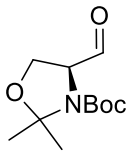


To a suspension of $LiAlH_4$ (2.11 g, 56.0 mmol) in anhydrous diethyl ether (140 mL), methyl ester **265** (7.23 g, 28.0 mmol) in anhydrous diethyl ether (140 mL) was added slowly portion by portion and stirred at room temperature overnight. The reaction was monitored by TLC. It was cooled to 0 °C and quenched by slow addition of 1.5 mL distilled water, 1.5 mL of 15% NaOH aqueous solution and then 6 mL distilled water. The reaction mixture was stirred at room temperature for 1 hour, filtered and washed with generous diethyl ether. The collected organic phases

were dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to afford **266** (5.25 g, 22.8 mmol, 81%) as yellow pale oil.

TLC (cyclohexane/ethyl acetate = 3:2) R_f =0.35 (Ninhydrin Stain). **IR** (ATR) $\tilde{\nu}$ = 3432 (w), 2978 (w), 2936 (w), 2879 (w), 1694 (m), 1671 (m), 1477 (w), 1457 (w), 1387 (s), 1365 (s), 1297 (w), 1257 (m), 1207 (w), 1170 (m), 1154 (m), 1105 (m), 1086 (m), 1071 (w), 1046 (m), 957 (w), 847 (m), 807 (w), 770 (w), 730 (m), 671 (w), 562 (w), 519 (w), 460 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 201 (2.93) nm. **MS** (EI, 70 eV): *m/z* (%) = 216 (24) $[M\text{-}CH_3]^+$, 200 (22), 160 (25), 158 (9), 144 (24), 117 (5), 116 (78), 101 (6), 100 (85), 83 (15), 59 (16), 58 (16), 57 (100), 56 (16), 43 (10), 42 (8), 41 (27), 39 (5). **GC** (HP-5MS): *I* = 1436. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 4.16-3.62 (m, 5H, CH, 2 x CH2), 3.53 (s, 1H, OH), 1.5 (s, 6H, 2 x CH_3), 1.44 (s, 9H, 3 x CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 154.0 (C), 94.1 (C), 81.2 (C), 65.3 (CH_2), 64.7 (CH_2), 59.5 (CH), 28.5 (3 x CH_3), 27.2 (CH_3), 24.6 (CH_3) ppm. **Optical rotation:** $[\alpha]_D^{20}$ = -1.8 (*c* 0.9, ethanol). **HRMS-ESI**: calcd. for $C_{10}H_{18}NO_4^+$ 216.1236; found 216.1238.

4.2.41 (*S*)-*tert*-Butyl 4-formyl-2,2-dimethyloxazolidine-3-carboxylate (260)

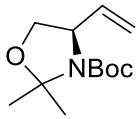


To a solution of oxalyl chloride (3.53 g, 28.0 mmol) in anhydrous dichloromethane (43 mL), dimethyl sulfoxide (4.39 g, 56.0 mmol) in anhydrous dichloromethane (5.4 mL) was added dropwise at -78 °C and stirred at this temperature for 30 minutes. Afterwards, **266** (4.31 g, 18.7 mmol) in anhydrous dichloromethane (32 mL) was added dropwise at -

78 °C and stirred at this temperature for another 30 minutes. After that, *N,N*-diisopropylethylamine (14.0 g, 108.2 mmol) was added and warmed slowly to 0 °C. 1M HCl (70 mL) ice-cold solution was used to quench the reaction mixture. The aqueous phase was extracted with dichloromethane. The combined organic layers were washed with pH 7.7 phosphate buffer, dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to obtain **260** (3.94 g, 17.2 mmol, 92%, *dr* 63:17) as colourless bright oil.

TLC (cyclohexane/ethyl acetate = 4:1) R_f = 0.25 (Ninhydrin Stain). **IR** (ATR) $\tilde{\nu}$ = 2979 (w), 2936 (w), 2280 (w), 1704 (s), 1478 (w), 1457 (w), 1365 (s), 1297 (w), 1258 (w), 1233 (w), 1165 (m), 1090 (m), 1059 (m), 1018 (w), 950 (w), 923 (w), 847 (m), 810 (m), 771 (w), 700 (w), 562 (w), 517 (w), 498 (s), 462 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 201 (3.34) nm. **MS** (EI, 70 eV): *m/z* (%) = 200 (8) $[M\text{-}CO]^+$, 156 (7), 144 (10), 114 (9), 101 (3), 100 (39), 98 (3), 86 (2), 84 (5), 83 (9), 70 (3), 59 (8), 58 (11), 57 (100), 56 (8), 55 (3), 44 (3), 43 (7), 42 (5), 41 (22), 39 (5). **GC** (HP-5MS): *I* = 1337. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = **A**: 9.53 (d, $^3J_{H,H}$ = 2.6 Hz, 1H, CH), 4.41-4.14 (m, 1H, CH), 4.06 (d, $^3J_{H,H}$ = 5.2 Hz, 2H, CH_2), 1.66-1.52 (m, 6H, 2 x CH_3), 1.52-1.38 (m, 12H, 3 x CH_3) ppm.; **B**: 9.59 (d, $^3J_{H,H}$ = 1.9 Hz, 1H, CH), 4.41-4.14 (m, 1H, CH), 4.06 (d, $^3J_{H,H}$ = 5.2 Hz, 2H, CH_2), 1.66-1.52 (m, 6H, 2 x CH_3), 1.52-1.38 (m, 9H, 3 x CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = **A**: 199.6 (CH), 111.5 (C), 95.2(C), 81.2 (C), 64.8 (CH), 64.1(CH_2), 28.4 (3 x CH_3), 25.9 (CH_3), 23.9 (CH_3) ppm.; **B**: 199.6 (CH), 11.4 (C), 94.5 (C), 81.5 (C), 64.9 (CH), 63.5 (CH_2), 28.4 (3 x CH_3), 26.6 (CH_3), 24.8 (CH_3) ppm. **Optical rotation**: $[\alpha]_D^{20}$ = -4.3 (*c* 0.4, ethanol).

4.2.42 (*R*)-*tert*-Butyl 2,2-dimethyl-4-vinyloxazolidine-3-carboxylate (267)

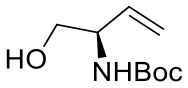


To a suspension of methyltriphenylphosphonium bromide (24.21 g, 67.91 mmol) in THF (590 mL), potassium bis(trimethylsilyl)amide (92.8 mL, 65.0 mmol, 0.7 M in toluene) was added and stirred at room temperature for 1 hour, subsequently cooled to -78 °C and a solution of Garner's aldehyde **260** (8.86 g, 38.7 mmol) in THF (118 mL) was put into the reaction mixture dropwise. It was warmed to room temperature slowly over 2 hours, then quenched with methanol (59 mL) and poured directly into a mixture of saturated potassium sodium tartrate and distilled water (1:1, 1180 mL). The aqueous phase was extracted with ethyl acetate, while the collected organic layers were dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to generate **267** (7.46 g, 32.9 mmol, 85%) as yellow bright oil.

TLC (cyclohexane/ethyl acetate = 10:1) R_f = 0.32 (Ninhydrin Stain). **IR** (ATR) $\tilde{\nu}$ = 2979 (w), 2935 (w), 2872 (w), 1693 (s), 1478 (w), 1456 (w), 1375 (s), 1364 (s), 1253 (m), 1206 (w), 1173 (m), 1141 (w), 1087 (s), 1059 (m), 983 (w), 919 (w), 860 (w), 840 (w), 806 (w), 768 (w), 743 (w), 718 (w), 670 (w), 515 (w), 462 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 201 (3.10) nm. **MS** (EI, 70 eV): *m/z* (%) = 212 (18) $[M\text{-}CH_3]^+$, 157 (6), 156 (78), 154 (4), 112 (36), 96 (8), 70 (15), 69 (4), 59 (8), 58 (5), 57 (100), 56 (11), 55 (4), 53 (3), 44 (5), 43 (5), 42 (7), 41 (26), 39 (7). **GC** (HP-5MS): *I* = 1276. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 5.91-5-59 (m, 1H, CH), 5.36-5.01 (m, 2H, CH_2), 4.49-4.20 (m, 1H, CH), 4.02 (dd, $^3J_{H,H}$ = 8.9, 6.2 Hz,

1H, CH_2), 3.72 (dd, $^4J_{H,H}$ = 2.3 Hz, $^3J_{H,H}$ = 8.9 Hz, 1H, CH_2), 1.58 (s, 6H, 2 x CH_3), 1.51-1.38 (m, 9H, 3 x CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 152.1 (C), 137.5 (CH), 115.9 (CH_2), 68.2 (CH_2), 59.8 (CH), 28.5 (5 x CH_3) ppm. **Optical rotation**: $[\alpha]_D^{20}$ = +0.9 (*c* 0.7, ethanol).

4.2.43 (*R*)-*tert*-Butyl (1-hydroxybut-3-en-2-yl)carbamate (268)

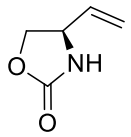


To a solution of vinyl oxazolidinone **267** (100 mg, 0.44 mmol) in methanol (4.4 mL), *p*-toluenesulfonic acid (42 mg, 0.22 mmol) was added at 0 °C, warmed to room temperature slowly and stirred overnight. Then, it was washed with saturated $NaHCO_3$ aqueous solution, extracted with diethyl ether, dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to afford **268** (62 mg, 0.36 mmol, 82%) as colourless oil.

TLC (cyclohexane/ethyl acetate = 1:1) R_f = 0.35 (Ninhydrin Stain). **IR** (ATR) $\tilde{\nu}$ = 3334 (w), 3085 (w), 2978 (w), 2932 (w), 1683 (s), 1504 (m), 1456 (w), 1391 (m), 1366 (m), 1248 (m), 1162 (s), 1048 (m), 990 (m), 920 (m), 878 (m), 850 (w), 781 (w), 597 (w), 461 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 201 (2.70) nm. **MS** (EI, 70 eV): *m/z* (%) = 156 (8) $[M\text{-}H_2O]^+$, 114 (8), 101 (18), 100 (46), 59 (25), 58 (5), 57 (100), 56 (90), 55 (13), 54 (4), 44 (15), 43 (8), 42 (5), 41 (59), 40 (6), 39 (19). **GC** (HP-5MS): *I* = 1319. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 5.79 (ddd, $^3J_{H,H}$ = 17.5, 10.5, 5.3 Hz,1H, CH), 5.32-5.16 (m, 2H, CH_2), 5.01 (d, $^3J_{H,H}$ = 8.8 Hz, 1H, CH), 4.22 (d, $^3J_{H,H}$ = 8.7 Hz, 1H, CH), 3.73-3.55 (m, 2H, CH_2), 2.58 (s, 1H, OH), 1.43 (s, 9H, 3 x CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 156.2

(C), 135.6 (CH), 116.6 (CH_2), 80.0 (C), 65.2 (CH_2), 54.8 (CH), 28.5 (3 x CH_3) ppm. **Optical rotation**: $[\alpha]_D^{20}$ = +1.2 (*c* 1.1, ethanol).

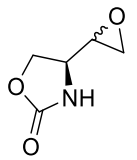
4.2.44 (*R*)-4-Vinyloxazolidin-2-one (269)



To a solution of **268** (0.65 g, 3.78 mmo) in anhydrous THF (75.6 mL, 0.05 mol/L), thionyl chloride (3.6 g, 30.24 mmol) was added dropwise at 0 °C, warmed to room temperature slowly and stirred at room temperature overnight. Then, the reaction mixture was concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to obtain **269** (350 mg, 3.1 mmol, 82%) as colourless oil.

TLC (cyclohexane/ethyl acetate = 1:1) R_f = 0.17 ($KMnO_4$). **IR** (ATR) $\tilde{\nu}$ = 3279 (m), 2987 (w), 2913 (w), 1726 (s), 1647 (w), 1542 (w), 1479 (w), 1396 (m), 1349 (w), 1319 (w), 1228 (m), 1127 (w), 1092 (w), 1047 (m), 1021 (m), 989 (m), 928 (m), 768 (m), 719 (m), 614 (m), 513 (m), 424 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 201 (2.89) nm. **MS** (EI, 70 eV): *m/z* (%) = 114 (34) $[M]^+$, 87 (20), 84 (38), 70 (14), 69 (100), 59 (8), 57 (12), 56 (74), 55 (78), 45 (16), 44 (10), 43 (66), 42 (41), 41 (11), 40 (31). **GC** (HP-5MS): *I* = 1214. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 6.23 (s, 1H, NH), 5.81 (ddd, $^3J_{H,H}$ = 15.5, 10.1, 7.3 Hz, 1H, CH), 5.34-5.22 (m, 2H, CH_2), 4.53 (td, $^3J_{H,H}$ = 8.5, 1.9 Hz, 1H, CH), 4.44-4.43 (m, 1H, CH_2), 4.05 (ddd, $^3J_{H,H}$ = 8.7, 6.6, 2.0 Hz, 1H, CH_2) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 160.0(C), 135.8 (CH), 118.7 (CH_2), 70.1 (CH_2), 55.4 (CH) ppm. **Optical rotation**: $[\alpha]_D^{20}$ = +2.0 (*c* 0.5, ethanol). **HRMS-ESI**: calcd. for $C_5H_7NO_2^+$ 113.0477; found 113.0476.

4.2.45 4-(Oxiran-2-yl)oxazolidin-2-one (270)

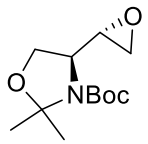


To a solution of olefine **269** (57 mg, 0.5 mmol) in dichloromethane (8.30 mL), *meta*-chloroperoxybenzoic acid (138 mg, 0.8 mmol) was added was added at 0 °C and stirred at room temperature for one week and washed with saturated $NaHCO_3$ aqueous solution untill *m*CPBA was consumed completely. It was extracted with diethyl ether, dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to afford **270** (38 mg, 0.3 mmol, 59%, *dr* 6:4) as colourless oil.

TLC (cyclohexane/ethyl acetate = 1:2) R_f = 0.1 ($KMnO_4$). **^{1}H NMR** (400 MHz, $CDCl_3$): δ = **A**: 6.76 (s, 1H, NH), 4.5 (td, $^2J_{H,H}$ = 8.9 Hz, $^3J_{H,H}$ = 2.9 Hz, 1H, CH_2), 4.28 (ddd, $^2J_{H,H}$ = 8.9 Hz, $^3J_{H,H}$ = 5.3, 3.7 Hz, 1H, CH), 3.83 (ddt, $^3J_{H,H}$ = 10.8, 8.9, 5.3 Hz, 1H, CH), 3.87-3.75 (m, 1H, CH), 2.82 (td, $^2J_{H,H}$ = 4.3 Hz, $^3J_{H,H}$ = 1.1 Hz, 1H, CH_2), 2.67 (td, $^2J_{H,H}$ = 4.6 Hz, $^3J_{H,H}$ = 2.6 Hz, 1H, CH_2) ppm.; **B**: 6.58 (s, 1H, NH), 4.5 (td, $^2J_{H,H}$ = 8.9 Hz, $^3J_{H,H}$ = 2.9 Hz, 1H, CH_2), 4.28 (ddd, $^2J_{H,H}$ = 8.9 Hz, $^3J_{H,H}$ = 5.3, 3.7 Hz, 1H, CH), 3.83 (ddt, $^3J_{H,H}$ = 10.8, 8.9, 5.3 Hz, 1H, CH), 3.87-3.75 (m, 1H, CH), 2.82 (td, $^2J_{H,H}$ = 4.3 Hz, $^3J_{H,H}$ = 1.1 Hz, 1H, CH_2), 2.67 (td, $^2J_{H,H}$ = 4.6 Hz, $^3J_{H,H}$ = 2.6 Hz, 1H, CH_2) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = **A**: 160.0 (C), 67.3 (CH), 53.8 (CH_2), 52.7 (CH_2), 44.6 (CH) ppm.; **B**: 160.0 (C), 66.7 (CH), 53.1 (CH_2), 52.3 (CH_2), 44.5 (CH) ppm. **HRMS-ESI**: calcd. for $C_5H_7NO_3^+$ 129.0426; found 129.0425.

4.2.46 Epoxidation of Compound 267

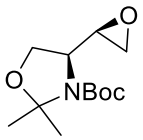
To a solution of olefin **267** (6.17 g, 27.19 mmol) in dichloromethane (453 mL, 0.06 mol/L), *meta*-chloroperoxybenzoic acid (13.41 g, 54.38 mmol, 70-75%) was added at 0 °C and stirred at room temperature for one week and washed with saturated $NaHCO_3$ aqueous solution untill *m*CPBA was consumed completely. It was extracted with diethyl ether, dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to afford **280** (3.48 g, 14.34 mmol, 53%) as white solid and **281** (1.74 g, 7.16 mmol, 26%) as white solid as well.



280

TLC (cyclohexane/ethyl acetate = 5:1) R_f = 0.29 (Ninhydrin Stain). **IR** (ATR) $\tilde{\nu}$ = 2979 (w), 2934 (w), 2878 (w), 1693 (s), 1478 (w), 1457 (w), 1375 (s), 1364 (s), 1320 (w), 1298 (w), 1246 (m), 1208 (w), 1166 (m), 1100 (w), 1083 (m), 1057 (m), 1012 (w), 968)w), 947 (w), 906 (m), 850 (m), 828 (m), 769 (m), 730 (w), 677 (w), 660 (w), 566 (w), 519 (w), 493 (w), 462 (w) cm^{-1}. **MS** (EI, 70 eV): *m/z* (%) = 228 (9) $[M\text{-}CH_3]^+$, 208 (5), 207 (24), 172 (11), 156 (5), 128 (57), 112 (7), 84 (7), 69 (6), 58 (6), 57 (100), 56 (38), 55 (13), 44 (44), 43 (18), 42 (11), 41 (64), 40 (40), 39 (21). **GC** (HP-5MS): *I* = 1462. **UV-Vis** (EtOH) λ_{max} (log ε) 201 (3.35) nm. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = **A**: 4.09-3.93 (m, 2H, CH_2), 3.60-3.35 (m, 1H, CH), 5.34-5.22 (m, 2H, CH_2), 3.05-2.85 (m, 2H, CH_2), 2.85-2.75 (m, 1H, CH), 1.61 (s, 6H, 2 x CH_3), 1.47 (s, 9H, 3 x CH_3) ppm. **B**: 4.09-3.93 (m, 2H, CH_2), 3.60-3.35 (m, 1H, CH), 5.34-5.22 (m, 2H, CH_2), 3.05-2.85 (m, 2H, CH_2), 2.85-2.75 (m, 1H, CH), 1.58 (s, 6H, 2 x CH_3), 1.45 (s, 9H, 3

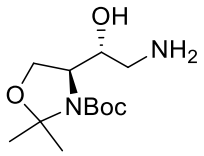
x CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = **A**: 152.5 (C), 94.6 (C), 80.3 (C), 66.3 (CH_2), 59.2 (CH), 52.6 (CH), 48.6 (CH_2), 28.6 (3 x CH_3), 26.8 (CH_3), 23.3 (CH_3) ppm.; **B**: 152.0 (C), 94.0 (C), 80.6 (C), 65.7 (CH_2), 59.5 (CH), 52.2 (CH), 48.4 (CH_2), 28.5 (3 x CH_3), 27.6 (CH_3), 24.5 (CH_3) ppm. **Optical rotation**: $[\alpha]_D^{20}$ = +1.3 (*c* 0.3, ethanol). **HRMS-ESI**: calcd. for $C_{11}H_{18}NO_4^+$ 228.1237; found 228.1237.



281

TLC (Cyclohexane: Ethyl acetate = 5:1) R_f = 0.23 (Ninhydrin Stain). **IR** (ATR) $\tilde{\nu}$ = 2987 (w), 2935 (w), 2884 (w), 2884 (w), 1696 (s), 1478 (w), 1459 (w), 1410 (w), 1376 (m), 1362 (m), 1293 (w), 1251 (w), 1230 (w), 1211 (w), 1162 (w), 1137 (w), 1101 (w), 1084 (m), 1056 (w), 1023 (w), 992 (w), 970 (w), 946 (w), 897 (w), 861 (w), 848 ()w), 827 (w), 806 (w), 791 (w), 768 (m), 739 (w), 680 (w), 567 (w), 517 (w), 463 (w), 446 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 201 (3.92) nm. **MS** (EI, 70 eV): *m/z* (%) = 228 (14) $[M\text{-}CH_3]^+$, 172 (13), 170 (6), 129 (6), 128(77), 112 (8), 84 (5), 69 (6), 59 (5), 57 (100), 56 (28), 55 (9), 44 (13), 43 (10), 42 (9), 41 (48), 40 (5), 39 (14). **GC** (HP-5MS): *I* = 1469.**^{1}H NMR** (400 MHz, $CDCl_3$): δ = 4.38-4.07 (m, 1H, CH), 3.96-3.62 (m, 2H, CH_2), 3.18 (dd, $^3J_{H,H}$ = 14.1 Hz, $^2J_{H,H}$ = 8.4 Hz, 1H, CH_2), 2.72 (t, $^3J_{H,H}$ = 4.4 Hz, 1H, CH), 2.62 (d, $^2J_{H,H}$ = 8.5 Hz, 1H, CH_2), 1.60-1.50 (m, 6H, 2 x CH_3), 1.47 (s, 9H, 3 x CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = **A**: 151.7 (C), 94.3 (C), 80.7 (C), 63.1 (CH_2), 56.5 (CH), 51.2 (CH), 44.3 (CH_2), 28.5 (3 x CH_3), 26.6 (CH_3), 23.3 (CH_3) ppm.; **B**: 152.0 (C), 93.8 (C), 80.3 (C), 63.2 (CH_2), 56.5 (CH), 51.2 (CH), 44.3 (CH_2), 28.5 (3 x CH_3), 27.2 (CH_3), 24.4 (CH_3) ppm. **Optical rotation**: $[\alpha]_D^{20}$ = 0.0 (*c* 0.1, ethanol). **HRMS-ESI**: calcd. for $C_{11}H_{18}NO_4^+$ 228.1237; found 228.1234.

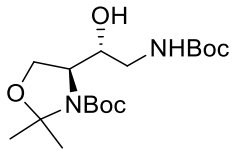
4.2.47 (*S*)-*tert*-Butyl 4-((*R*)-2-amino-1-hydroxyethyl)-2,2-dimethyloxazolidine-3-carboxylate (282)



To 25% aqueous ammonia (107 mL), a solution of epoxide **280** (1.3 g, 5.35 mmol) in ethanol (11 mL) was added rapidly and stirred at room temperature overnight. Then, a solution of aqueous 1N NaOH (214 mL) was put into the reaction mixture and extracted with dichloromethane. The collected organic phases were dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to afford **282** (1.34 g, 5.14 mmol, 96%) as white solid.

TLC (chloroform/methanol/ammonia = 80:10:1) R_f = 0.24 (Ninhydrin Stain). **IR** (ATR) $\tilde{\nu}$ = 3373 (w), 3297 (w), 2979 (w), 2937 (w), 2875 (w), 2737 (w), 1683 (s), 1588 (w), 1478 (w), 1460 (w), 1362 (s), 1311 (w), 1284 (w), 1254 (w), 1206 (m), 1167 (w), 1146 (w), 1116 (w), 1088 (w), 1074 (w), 1045 (w), 1035 (w), 994 (w), 978 (w), 943 (w), 920 (w), 849 (w), 807 (w), 763 (w), 728 (w), 728 (w), 681 (w), 663 (w), 640 (w), 564 (w), 528 (w), 483 (w), 461 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 201 (3.52) nm. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 4.22-3.99 (m, 1H, CH), 3.96-3.85 (m, 2H, CH_2), 3.60-3.45 (m, 1H, CH), 2.85-2.65 (m, 4H, CH_2, NH_2), 1.51 (s, 3H, CH_3), 1.47 (s, 12H, 4 x CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 154.0 (C), 94.1 (C), 81.0 (C), 71.9 (CH), 65.5 (CH_2), 59.5 (CH), 43.1 (CH_2), 28.5 (3 x CH_3), 27.4 (CH_3), 24.3 (CH_3) ppm. **Optical rotation**: $[\alpha]_D^{20}$ = +0.5 (*c* 0.2, ethanol).

4.2.48 (*S*)-*tert*-Butyl 4-((*R*)-2-((*tert*-butoxycarbonyl)amino)-1-hydroxyethyl)-2,2-dimethyloxazolidine-3-carboxylate (283)

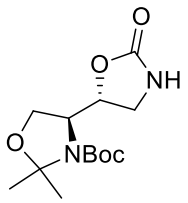


To a solution of amino alcohol **282** (2.43 g, 9.36 mmol) in 1N NaOH aqueous solution, a solution of di-*tert*-butyl dicarbonate (2.45 g, 11.23 mmol) in 1,4-dioxane (8.8 mL) was added at 0 °C and stirred at this temperature for 30 minutes. Then, it was warmed to room temperature and stirred at room temperature overnight. It was acidified to pH 2-3 by addition of 1N potassium bisulfate aqueous solution, extracted with ethyl acetate. The combined organic phases were dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to produce **283** (3.3 g, 9.17 mmol, 95%) as white solid.

TLC (cyclohexane/ethyl acetate = 3:1) R_f = 0.26 (Ninhydrin Stain). **IR** (ATR): 3321 (w), 3231 (w), 2974 (w), 2915 (w), 2884 (w), 1691 (m), 1666 (m), 1552 (m), 1457 (w), 1422 (w), 1377 (m), 1362 (m), 1349 (w), 1302 (m), 1273 (w), 1252 (m), 1170 (w), 1150 (m), 1114 (w), 1093 (w), 1080 (m), 1066 (m), 1042 (w), 979 (w), 951 (w), 924 (w), 881 (m), 846 (w), 846 (w), 806 (w), 789 (w), 763 (w), 726 (w), 656 (w), 578 (w), 561 (w), 525 (w), 510 (w), 482 (w), 463 (w), 445 (w), 422 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 201 (3.62) nm. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 5.73 (s, 1H, NH), 4.15 (s, 1H, OH), 4.13-3.09 (m, 1H, CH), 4.03-3.89 (m, 2H, CH_2), 3.69-3.46 (m, 2H, CH_2), 2.97-2.81 (m, 1H, CH), 1.51 (s, 3H, CH_3), 1.48 (s, 12H, 4 x CH_3), 1.43 (s, 9H, 3 x CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 158.4 (C), 154.3 (C), 94.2 (C), 81.2 (C), 79.8 (C), 72.5 (CH), 65.1

(CH_2), 59.1 (CH), 43.1 (CH_2), 28.5 (3 x CH_3), 28.4 (3 x CH_3), 27.5 (CH_3), 24.3 (CH_3) ppm. **Optical rotation**: $[\alpha]_D^{20}$ = -7.1 (*c* 0.1, ethanol).

4.2.49 (4*S*,5'*R*)-*tert*-Butyl 2,2-dimethyl-2'-oxo-[4,5'-bioxazolidine]-3-carboxylate (278)

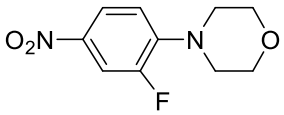


To a solution of *N*-Boc amino alcohol **283** (1.26 g, 3.51 mmol) in anhydrous THF (35 mL), sodium hydride (168 mg, 4.21 mmol, 60% in mineral oil) was added and stirred under reflux for 3 hours. Then, it was cooled to room temperature, washed with saturated ammonium chloride aqueous solution, extracted with dichloromethane, dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to generate **278** (0.87 g, 3.02 mmol, 86%) as white solid.

TLC (cyclohexane/ethyl acetate = 1:2) R_f = 0.32 (Ninhydrin Stain). **IR** (ATR) $\tilde{\nu}$ = 3315 (w), 3008 (w), 2972 (w), 2905 (w), 1752 (w), 1715 (m), 1694 (m), 1477 (w), 1456 (w), 1440 (w), 1392 (w), 1362 (m), 1339 (m), 1301 (w), 1287 (w), 1249 (w), 1233 (w), 1209 (w), 1170 (w), 1155 (w), 1097 (w), 1052 (w), 1028 (w), 987 (w), 967 (w), 947 (w), 921 (w), 874 (w), 784 (w), 765 (w), 748 (w), 701 (w), 653 (w), 615 (w), 566 (w), 544 (w), 516 (w), 465 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 201 (3.91) nm. **MS** (EI, 70 eV): *m/z* (%) = 213 (8) $[M\text{-}OCH_3]^+$, 207 (11), 200 (28), 171 (47), 155 (11), 144 (20), 101 (7), 100 (66), 98 (6), 87 (41), 84 (6), 83 (10), 68 (9), 59 (7), 58 (10), 57 (100), 56 (21), 55 (7), 44 (10), 43 (8), 42 (9), 41 (31), 39 (11). **GC** (HP-5MS): *I* = 2124. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 5.94 (s, 1H, NH), 4.80-4.55 (m, 1H, CH), 4.30-4.01 (m, 2H, CH_2), 3.95

(dd, $^{3}J_{H,H}$ = 9.5, 5.5 Hz, 1H, CH), 3.80-3.49 (m, 2H, CH_2), 1.54 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 1.46 (s, 9H, 3 x CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 159.7 (C), 153.4 (C), 94.4 (C), 81.2 (C), 76.3 (CH), 64.5 (CH_2), 59.0 (CH), 43.6 (CH_2), 28.4 (3 x CH_3), 27.7 (CH_3), 24.5 (CH_3) ppm. **Optical rotation**: $[\alpha]_D^{20}$ = -8.8 (*c* 0.1, ethanol).

4.2.50 4-(2-Fluoro-4-nitrophenyl)morpholine (275)

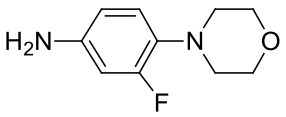


To a solution of morpholine (21.57 g, 247.6 mmol) and *N,N*-diisopropylethylamine (32 g, 247.6 mmol) in ethyl acetate (130 mL), 3.4-difluoronitrobenzene (35.92 g, 225.8 mmol) was added slowly via an additional funnel. After adding two-thirds, the reaction was warmed higher than 35 °C. Subsequently, it was cooled in an ice bath. The rest of 3,4-difluoronitrobenzene was added over 30 minutes. The reaction mixture was warmed to room temperature and stirred at this temperature overnight. Then, dichloromethane (100 mL), ethyl acetate (400 mL) and distilled water (100 mL) were added to the reaction, in which yellow solid was produced. The aqueous phase was extracted with ethyl acetate. The collected organic layers were dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and recrystallized from acetone and water to affford 4-(2-fluoro-4-nitrophenyl)morpholine **275** (45.85 g, 202.88 mmol, 90%) as yellow crystals.

IR (ATR) $\tilde{\nu}$ = 3050 (w), 2962 (w), 2906 (w), 2878 (w), 2851 (w), 1601 (m), 1514 (m), 1492 (m), 1466 (w), 1444 (w), 1383 (w), 1361 (w), 1324 (m), 1272 (m), 1261 (m), 1222 (m), 1213 (m), 1122 (m), 1089 (m), 1072 (m), 1048 (m), 1027 (m), 947 (m), 915 (m), 858 (w), 848 (w), 815 (w), 802 (m), 745 (m), 711 (m), 653 (m), 601 (w), 575 (w), 557 (w), 534 (w), 475

(w), 419 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 354 (3.12), 201 (3.47) nm. **MS** (EI, 70 eV): *m/z* (%) = 227 (9) $[M+H]^+$, 226 (80), 225 (11), 210 (5), 196 (5), 169 (9), 168 (100), 167 (5), 138 (22), 122 (13), 121 (8), 102 (7), 95 (13), 94 (7), 75 (6). **GC** (HP-5MS): *I* = 1916. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 7.97 (ddd, $^4J_{H,H}$ = 2.6, 1.1Hz, $^3J_{H,H}$ = 9.0 Hz, 1H, CH), 7.88 (dd, $^4J_{H,H}$ = 2.6 Hz, $^3J_{H,H}$ = 13.1 Hz, 1H, CH), 6.91 (t, $^3J_{H,H}$ = 8.8 Hz, 1H, CH), 3.89-3.83 (m, 4H, 2 x CH_2), 3.30-3.25 (m, 4H, 2 x CH_2) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 153.2 (d, $^1J_{C,F}$ = 249.5 Hz, C), 145.6 (d, $^2J_{C,F}$ = 7.7 Hz, C), 131.4 (d, $^3J_{C,F}$ = 8.1 Hz, CH), 121.1 (d, $^4J_{C,F}$ = 3.0 Hz, CH), 117.0 (d, $^3J_{C,F}$ = 3.9 Hz, CH), 112.7 (d, $^2J_{C,F}$ = 26.4 Hz, C), 66.7 (2 x CH_2), 50.0 (d, $^4J_{C,F}$ = 5.0 Hz, 2 x CH_2) ppm. **^{19}F NMR** (400 MHz, $CDCl_3$): δ = -119.0 ppm.

4.2.51 3-Fluoro-4-morpholinoaniline (72)

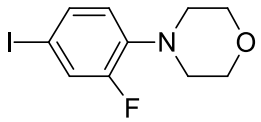


To a solution of 4-(2-fluoro-4-nitrophenyl)morpholine (7.79 g, 34.5 mmol) and cobalt(II) chloride hexahydrate (16.42 g, 69.0 mmol) in methanol (206 mL), sodium borohydride (13.04 g, 345 mmol) was added in portions with stirring at 60 °C overnight. Then, 3N hydrochloric acid was added and extracted with diethyl ether. The combined organic layers were dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to produce 3-fluoro-4-morpholinoaniline **72** (4.85 g, 24.7 mmol, 72%) as brown solid.

TLC (cyclohexane/ethyl acetate = 1:1) R_f = 0.35 (UV). **IR** (ATR) $\tilde{\nu}$ = 3415 (w), 3333 (w), 3229 (w), 2971 (w), 2938 (w), 2897 (w), 2864 (w), 2822 (w), 1638 (w), 1577 (w), 1511 (m), 1478 (w), 1448 (w), 1375 (w), 1335

(w), 1316 (w), 1300 (w), 1271 (w), 1248 (w), 1220 (w), 1205 (w), 1160 (w), 1108 (m), 1043 (w), 1026 (w), 961 (w), 926 (w), 915 (w), 843 (w), 814 (w), 741 (w), 725 (w), 711 (w), 653 (w), 626 (w), 613 (w), 580 (w), 516 (w), 495 (w), 464 (w), 431 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 301 (2.79), 247 (3.57), 203 (3.74) nm. **MS** (EI, 70 eV): *m/z* (%) = 197 (61) $[M+H]^+$, 196 (100), 195 (41), 151 (13), 139 (40), 138 (100), 137 (88), 136 (6), 124 (25), 117 (23), 111 (6), 110 (16), 109 (11), 90 (12), 83 (33), 68 (12), 57 (10). **GC** (HP-5MS): *I* = 1721. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 6.85-6.71 (m, 1H, CH), 6.48-6.35 (m, 2H, 2 x CH), 4.05-3.71 (m, 4H, 2 x CH_2), 3.73-3-33 (m, 2H, NH_2), 3.05-2.86 (m, 4H, 2 x CH_2) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 156.8 (d, $^1J_{C,F}$ = 245.2 Hz, C), 143.0 (d, $^2J_{C,F}$ = 10.5 Hz, C), 131.8 (d, $^3J_{C,F}$ = 9.6 Hz, CH), 120.4 (d, $^4J_{C,F}$ = 4.4 Hz, CH), 110.7 (d, $^3J_{C,F}$ = 3.0 Hz, CH), 104.0 (d, $^2J_{C,F}$ = 23.7 Hz, C), 67.2 (2 x CH_2), 51.8 (d, $^4J_{C,F}$ = 2.4 Hz, 2 x CH_2) ppm. **^{19}F NMR** (400 MHz, $CDCl_3$): δ = -123.1 ppm.

4.2.52 4-(2-Fluoro-4-iodophenyl)morpholine (279)

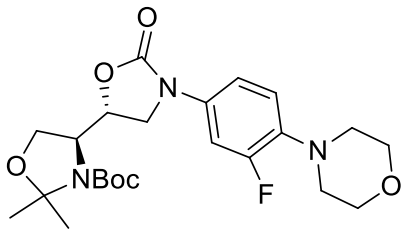


To a solution of *p*-toluenesulfonic acid monohydrate (571 mg, 3.0 mmol) in acetonitrile (2.0 mL), 3-fluoro-4-morpholinoaniline (196 mg, 1.0 mmol) was added. Then, it was cooled to 10-15 °C, a solution of sodium nitrite (138 mg, 2.0 mmol) and potassium iodide (415 mg, 2.5 mmol) in distilled water (0.5 mL) was added slowly to the suspension and stirred for 10 minutes. It was warmed to room temperature and stirred at room temperature till the starting material was completely consumed. To the reaction mixture, distilled water, sodium bicarbonate and sodium thiosulfate were added, extracted with ethyl acetate. The collected

organic phases were washed with distilled water and birne, dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to create 4-(2-fluoro-4-iodophenyl)morpholine **279** (297 mg, 0.97 mmol, 97%) as yellow solid.

TLC (cyclohexane/ethyl acetate = 5:1) R_f = 0.39 (UV). **IR** (ATR) $\tilde{\nu}$ = 3004 (w), 2961 (w), 2892 (w), 2851 (w), 1719 (w), 1679 (w), 1597 (w), 1558 (w), 1487 (m), 1450 (w), 1401 (w), 1372 (w), 1333 (w), 1295 (w), 1251 (w), 1234 (w), 1201 (w), 1165 (w), 1111 (m), 1071 (w), 1045 (w), 1030 (w), 919 (w), 859 (w), 852 (w), 842 (w), 802 (m), 725 (w), 646 (w), 572 (w), 496 (w), 470 (w), 422 (w) cm^{-1}. **MS** (EI, 70 eV): *m/z* (%) = 309 (46) $[M+2H]^+$, 308 (76), 307 (73), 306 (16), 250 (54), 249 (100), 248 (99), 235 (18), 221 (14), 136 (10), 125 (25), 124 (23), 123 (50), 122 (52), 110 (13), 109 (19), 108 (12), 103 (30), 97 (19), 96 (65), 95 (79), 94 (10), 82 (11), 77 (15), 76 (42), 75 (11), 69 (11), 58 (28), 52 (11), 51 (13), 43 (22). **GC** (HP-5MS): *I* = 1819. **UV-Vis** (EtOH) λ_{max} (log ε) 255 (3.79), 201 (3.95) nm. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 7.40-7.30 (m, 2H, 2 x CH), 6.66 (d, $^3J_{H,H}$ = 8.9 Hz, 1H, CH), 4.35-3.63 (m, 4H, 2 x CH_2), 3.22-2.90 (m, 4H, 2 x CH_2) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 156.8 (d, $^1J_{C,F}$ = 251.7 Hz, C), 140.1 (d, $^2J_{C,F}$ = 10.5 Hz, C), 133.7 (d, $^3J_{C,F}$ = 3.7 Hz, CH), 125.3 (d, $^2J_{C,F}$ = 23.4 Hz, CH), 120.4 (d, $^3J_{C,F}$ = 3.5 Hz, CH), 83.3 (d, $^3J_{C,F}$ = 8.3 Hz, C), 67.0 (2 x CH_2), 50.8 (d, $^4J_{C,F}$ = 3.5 Hz, 2 x CH_2) ppm. **^{19}F NMR** (400 MHz, $CDCl_3$): δ = -120.3 ppm.

4.2.53 (4*S*,5'*R*)-*tert*-Butyl 3'-(3-fluoro-4-morpholinophenyl)-2,2-dimethyl-2'-oxo-[4,5'-bioxazolidine]-3-carboxylate (293)

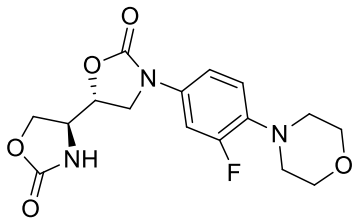


To a solution of oxazolidinone **278** (112 mg, 0.39 mmol) in 1,4-dioxane (3.9 mL), 4-(2-fluoro-4-iodophenyl)morpholine (120 mg, 0.39 mmol), copper iodide (4 mg, 0.02 mmol), caesium carbonate (254 mg, 0.78 mmol) were added, evacuated and backfilled with argon three times. Then, *N,N'*-dimethylethylenediamine (3 mg, 0.039 mmol, 10 mol%) was added, evacuated and backfilled with argon again. The reaction mixture was stirred at 110 °C for three hours and cooled to room temperature. It was diluted with ethyl acetate, filtered through Celite® and concentrated under reduced pressure using a rotary evaporator. The residue was purified by flash column chromatography on silica gel to generate **293** (178 mg, 0.38 mmol, 97%) as white solid.

TLC (cyclohexane/ethyl acetate = 2:1) R_f = 0.39 (UV). **IR** (ATR) $\tilde{\nu}$ = 2974 (w), 2932 (w), 2829 (w), 1738 (m), 1690 (m), 1630 (w), 1573 (w), 1486 (w), 1451 (w), 1423 (w), 1410 (w), 1387 (w), 1361 (m), 1348 (m), 1328 (w), 1287 (w), 1271 (w), 1234 (w), 1194 (w), 1168 (m), 1137 (w), 1114 (m), 1097 (w), 1080 (m), 1046 (m), 1021 (m), 935 (m), 921 (w), 882 (w), 863 (w), 843 (w), 824 (w), 803 (w), 765 (w), 751 (w), 736 (w), 659 (w), 614 (w), 580 (w), 567 (w), 541 (w), 515 (w), 468 (w), 449 (w), 427 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 258 (3.97), 201 (4.05) nm. **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 7.40 (dd, $^4J_{H,H}$ = 2.5 Hz, $^3J_{H,F}$ = 14.3 Hz, 1H, CH), 7.10 (d, $^3J_{H,H}$ = 8.9 Hz, 1H, CH), 6.91 (t, $^3J_{H,H}$ = 9.1 Hz, 1H, CH), 4.59 (q, $^3J_{H,H}$ = 7.8 Hz, 1H, CH), 4.30-4.01 (m, 3H, CH, CH_2), 4.04-3.93 (m, 2H,

CH_2), 3.85 (dd, $^4J_{H,H}$ = 3.5 Hz, $^3J_{H,H}$ = 5.8 Hz, 4H, 2 x CH_2), 3.03 (dd, $^3J_{H,H}$ = 5.8 Hz, $^4J_{H,H}$ = 3.5 Hz, 4H, 2 x CH_2), 1.64-1.32 (m, 15H, 5 x CH_3) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): δ = 155.6 (d, $^1J_{C,F}$ = 246.3 Hz, C), 154.3 (C), 153.6 (C), 136.5 (d, $^3J_{C,F}$ = 9.0 Hz, C), 133.4 (d, $^2J_{C,F}$ = 10.5 Hz, C), 118.9 (d, $^4J_{C,F}$ = 4.1 Hz, CH), 114.4 (CH), 107.9 (d, $^3J_{C,F}$ = 25.8 Hz, CH), 94.6 (C), 81.4 (C), 72.8 (CH), 67.1 (2 x CH_2), 64.5 (CH_2), 59.2 (CH), 51.1 (d, $^4J_{C,F}$ = 2.9 Hz, 2 x CH_2), 48.6 (CH_2), 27.8 (3 x CH_3), 27.8 (CH_3), 24.5 (CH_3) ppm. **^{19}F NMR** (400 MHz, $CDCl_3$): δ = -120.1, -120.6 ppm. **Optical rotation**: $[\alpha]_D^{20}$ = -9.4 (*c* 0.2, ethanol). **HRMS-ESI**: calcd. for $C_{23}H_{32}FN_3O_6^+$ 465.2275; found 465.2271.

4.2.54 (4*S*,5'*R*)-3'-(3-Fluoro-4-morpholinophenyl)-[4,5'-bioxazolidine]-2,2'-dione (92)



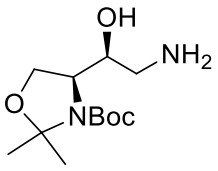
To a solution of oxazolidinone **293** (622 mg, 1.34 mmol) in dichloromethane (13 mL), 4N HCl in 1,4-dioxane (3.4 mL, 13.4 mmol) was added and stirred at room temperature overnight. It was concentrated under reduced pressure using a rotary evaporator and used for the next step without purification.

To a suspension of amino salts (1.34 mmol) in anhydrous THF (13 mL), *N*,*N*-diisopropylethylamine (434 mg, 3.35 mmol) was added till the salts were completely dissolved into the organic solvent. Then, triphosgene (400 mg, 1.34 mmol) in anhydrous THF was added at 0 °C over 1 hour and stirred at 0 °C for 2-3 hours. After that, it was warmed to room temperature and stirred overnight. The reaction mixture was washed with

distilled water, extracted with ethyl acetate, dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to yield **92** (409 mg, 1.17 mmol, 87% over two steps) as white solid.

TLC (ethyl acetate) R_f = 0.18 (UV). **IR** (ATR) $\tilde{\nu}$ = 3367 (w), 2976 (w), 2959 (w), 2942 (w), 2914 (w), 2853 (w), 2820 (w), 1750 (m), 1723 (m), 1625 (w), 1572 (w), 1514 (m), 1472 (w), 1446 (w), 1399 (w), 1376 (w), 1346 (w), 1325 (w), 1300 (w), 1272 (w), 1225 (m), 1166 (w), 1138 (w), 1120 (m), 1076 (w), 1050 (m), 1024 (w), 997 (w), 958 (w), 940 (w), 919 (w), 871 (m), 846 (w), 816 (m), 751 (m), 732 (m), 719 (w), 657 (w), 633 (w), 605 (w), 577 (w), 528 (w), 503 (w), 494 (w), 467 (w), 424 (w), 410 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 258 (3.50), 201 (3.92) nm. **^{1}H NMR** (500 MHz, DMSO-D_6): δ = 8.12 (s, 1H, NH), 7.48 (dd, $^4J_{H,H}$ = 2.5 Hz, $^3J_{H,F}$ = 15.0 Hz, 1H, CH), 7.17 (dd, $^4J_{H,H}$ = 2.5 Hz, $^3J_{H,H}$ = 8.8 Hz, 1H, CH), 7.07 (t, $^3J_{H,H}$ = 9.3 Hz, 1H, CH), 4.72 (ddd, $^3J_{H,H}$ = 8.8, 6.2, 4.3 Hz, 1H, CH), 4.53-4.27 (m, 1H, CH), 4.24-3.98 (m, 2H, CH_2), 3.79-3.74 (m, 2H, CH_2), 3.73 (dt, $^3J_{H,H}$ = 4.8, 3.0 Hz, 4H, 2 x CH_2), 3.02-2.86 (m, 4H, 2 x CH_2) ppm. **^{13}C NMR** (125 MHz, DMSO-D_6): δ = 157.6 (d, $^1J_{C,F}$ = 406.3 Hz, C), 154.1 (C), 154.0 (C), 136.2 (d, $^3J_{C,F}$ = 8.8 Hz, C), 133.7 (d, $^2J_{C,F}$ = 10.6 Hz, C), 119.8 (d, $^4J_{C,F}$ = 4.1 Hz, CH), 114.6 (d, $^4J_{C,F}$ = 3.1 Hz, CH), 107.2 (d, $^3J_{C,F}$ = 26.2 Hz, CH), 73.2 (CH), 66.6 (2 x CH_2), 65.3 (CH_2), 53.7 (CH), 51.2 (d, $^4J_{C,F}$ = 2.9 Hz, 2 x CH_2), 46.2 (CH_2) ppm. **^{19}F NMR** (500 MHz, DMSO-D_6): δ = -121.4 ppm. **Optical rotation**: $[\alpha]_D^{20}$ = -2.0 (*c* 0.2, ethanol). **HRMS-ESI**: calcd. for $C_{16}H_{19}FN_3O_5^+$ 352.1303; found 352.1303.

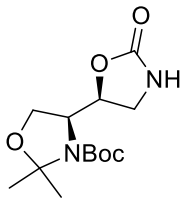
4.2.55 (*S*)-*tert*-Butyl 4-((*S*)-2-amino-1-hydroxyethyl)-2,2-dimethyloxazolidine-3-carboxylate (295)



To 25% aqueous ammonia (140 mL), a solution of epoxide **281** (1.7 g, 7.0 mmol) in ethanol (14 mL) was added rapidly and stirred at room temperature overnight. Then, a solution of aqueous 1N NaOH (280 mL) was put into the reaction mixture and extracted with dichloromethane. The collected organic phases were dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to afford **295** (1.31 g, 5.05 mmol, 72%) as white solid.

TLC (chloroform/methanol/ammonia = 80:10:1) R_f = 0.06 (Ninhydrin Stain). **IR** (ATR) $\tilde{\nu}$ = 3356 (w), 3287 (w), 3183 (w), 2980 (w), 2935 (w), 2877 (w), 1699 (m), 1685 (m), 1599 (w), 1476 (w), 1456 (w), 1386 (m) 1363 (m), 1348 (m), 1329 (m), 1308 (w), 1254 (w), 1230 (w), 1203 (w), 1170 (w), 1150 (w), 1111 (w), 1087 (w), 1066 (m), 1029 (m), 982 (w), 949 (w), 887 (w), 865 (w), 807 (w), 789 (w), 766 (w), 747 (w), 721 (w), 690 (w), 667 (w), 586 (w), 548 (w), 520 (w), 502 (w), 480 (w), 463 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 201 (3.02) nm. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 4.25-3.62 (m, 2H, CH_2), 2.99-2.53 (m, 1H, CH), 2.49-2.19 (m, 3H, CH, CH_2), 1.56 (s, 3H, CH_3), 1.47 (s, 12H, 4 x CH_3) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 154.7 (C), 94.2 (C), 81.3 (C), 74.2 (CH), 64.6 (CH_2), 60.6 (CH), 44.7 (CH_2), 28.5 (3 x CH_3), 27.1 (CH_3), 24.3 (CH_3) ppm. **Optical rotation**: $[\alpha]_D^{20}$ = -5.7 (*c* 0.2, ethanol). **HRMS-ESI**: calcd. for $C_{12}H_{24}N_2O_4Na^+$ 283.1628; found 283.1628.

4.2.56 (4*S*,5'*S*)-*tert*-Butyl 2,2-dimethyl-2'-oxo-[4,5'-bioxazolidine]-3-carboxylate (296)

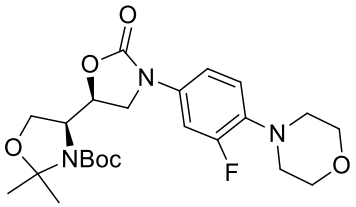


To a solution of amino alcohol **295** (809 mg, 3.11 mmol) and *N,N*-diisopropylethylamine (804 mg, 6.22 mmol) in anhydrous THF (31 mL), triphosgene (0.93 g, 3.11 mmol) in anhydrous THF (31 mL) was added at 0 °C over 1 hour and stirred at 0 °C for 2-3 hours. Then, it was stirred at room temperature overnight, washed with distilled water, extracted with ethyl acetate, dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to create **296** (653 mg, 2.29 mmol, 74%) as white solid.

TLC (cyclohexane/ethyl acetate = 1:2) R_f = 0.32 (Ninhydrin stain). **IR** (ATR) $\tilde{\nu}$ = 3269 (w), 2776 (w), 2932 (w), 1749 (w), 1707 (w), 1681 (m), 1499 (w), 1476 (w), 1437 (w), 1388 (m), 1363 (m), 1349 (w), 1315 (w), 1287 (m), 1258 (m), 1214 (w), 1169 (w), 1155 (w), 1087 (m), 1054 (m), 1012 (w), 968 (w), 951 (w), 936 (w), 880 (w), 859 (w), 843 (w), 806 (w), 770 (w), 743 (w), 719 (w), 666 (w), 565 (w), 533 (w), 521 (w), 459 (w), 424 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 201 (3.40) nm. **MS** (EI, 70 eV): *m/z* (%) = 213 (6) $[M\text{-}OCH_3]^+$, 207 (42), 200 (16), 171 (40), 144 (13), 100 (55), 98 (6), 87 (38), 86 (8), 83 (10), 58 (10), 57 (100), 56 (32), 44 (54), 41 (41), 39 (14). **GC** (HP-5MS): *I* = 2124. **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 4.71-4.55 (m, 1H, CH), 4.40-3.99 (m, 2H CH_2), 3.96 (dd, $^3J_{H,H}$ = 9.5, 5.5 Hz, 1H, CH), 3.80-3.50 (m, 2H, CH_2), 1.64-1.40 (m, 24H, 8 x CH_3) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): δ = 159.5 (C), 153.5 (C), 94.4 (C), 81.2 (C), 76.4 (CH), 64.5 (CH_2), 59.0 (CH), 43.6 (CH_2), 28.4 (3 x CH_3), 27.7 (CH_3),

24.5 (CH_3) ppm. **Optical rotation**: $[\alpha]_D^{20}$ = +1.6 (*c* 0.2, ethanol). **HRMS-ESI**: calcd. for $C_{13}H_{22}N_2O_5Na^+$ 309.1421; found 309.1421.

4.2.57 (4*S*,5'*S*)-*tert*-Butyl 3'-(3-fluoro-4-morpholinophenyl)-2,2-dimethyl-2'-oxo-[4,5'-bioxazolidine]-3-carboxylate (297)

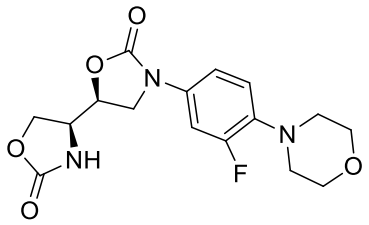


To a solution of oxazolidinone **296** (143 mg, 0.5 mmol) in 1,4-dioxane (5.0 mL), 4-(2-fluoro-4-iodophenyl)morpholine (154 mg, 0.5 mmol), copper iodide (5 mg, 0.025 mmol), caesium carbonate (326 mg, 1.0 mmol) were added, evacuated and backfilled with argon three times. Then, *N,N'*-dimethylethylenediamine (4 mg, 0.05 mmol, 10 mol%) was added, evacuated and backfilled with argon again. The reaction mixture was stirred at 110 °C for three hours and cooled to room temperature. It was diluted with ethyl acetate, filtered through Celite® and concentrated under reduced pressure using a rotary evaporator. The residue was purified over column chromatography on silica gel to generate **297** (229 mg, 0.49 mmol, 98%, *dr* 53:47) as white solid.

TLC (cyclohexane/ethyl acetate = 5:1) R_f = 0.31 (UV). **IR** (ATR) $\tilde{\nu}$ = 3071 (w), 3004 (w), 2961 (w), 2920 (w), 2892 (w), 2852 (w), 2689 (w), 1733 (w), 1597 (w), 1558 (w), 1487 (m), 1450 (w), 1401 (w), 1372 (w), 1334 (w), 1296 (w), 1259 (w), 1251 (w), 1233 (w), 1201 (m), 1165 (w), 1112 (m), 1072 (w), 1045 (w), 1031 (w), 919 (m), 859 (m), 842 (m), 802 (m), 726 (w), 647 (w), 572 (m), 496 (w), 470 (w), 424 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 255 (3.62), 206 (3.26) nm. **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 7.45 (dd, $^4J_{H,H}$ = 2.6 Hz, $^3J_{H,F}$ = 14.4 Hz, 1H, CH), 7.10 (dd, $^4J_{H,H}$ = 2.8 Hz,

$^{3}J_{H,H}$ = 8.9 Hz, 1H, CH), 6.98 (m, 1H, CH), 5.15-4.73(m, 1H, CH), 4.43-4.20 (m, 1H, CH), 4.21-3.96 (m, 4H, 2 x CH_2), 3.88 (q, $^{4}J_{H,H}$ = 4.6 Hz, $^{3}J_{H,H}$ = 6.0 Hz, 4H, 2 x CH_2), 3.11-3.00 (m, 4H, 2 x CH_2) 1.68-1.40 (m, 15H, 5 x CH_3) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): δ = **A**: 155.7 (d, $^{1}J_{C,F}$ = 246.6 Hz, C), 154.5 (C), 153.3 (C), 136.3 (C), 133.5 (C), 119.2 (CH), 114.1 (d, $^{4}J_{C,F}$ = 3.3 Hz, CH), 114.4 (CH), 107.7 (d, $^{3}J_{C,F}$ = 26.3 Hz, CH), 94.8 (C), 81.5 (C), 71.3 (CH), 67.0 (2 x CH_2), 63.5 (CH_2), 57.9 (CH), 51.1 (2 x CH_2), 46.4 (CH_2), 28.5 (3 x CH_3), 27.3 (CH_3), 23.9 (CH_3) ppm.; **B**: 155.7 (d, $^{1}J_{C,F}$ = 246.6 Hz, C), 154.4 (C), 152.0 (C), 136.3 (C), 133.5 (C), 119.2 (CH), 114.1 (d, $^{4}J_{C,F}$ = 3.3 Hz, CH), 114.4 (CH), 107.7 (d, $^{3}J_{C,F}$ = 26.3 Hz, CH), 95.1 (C), 81.3 (C), 71.3 (CH), 67.0 (2 x CH_2), 63.3 (CH_2), 57.6 (CH), 51.1 (2 x CH_2), 46.4 (CH_2), 28.4 (3 x CH_3), 26.8 (CH_3), 22.5 (CH_3) ppm. **^{19}F NMR** (500 MHz, $CDCl_3$): δ = -120.2, -120.3 ppm. **Optical rotation**: $[\alpha]_D^{20}$ = +1.6 (*c* 0.3, ethanol).

4.2.58 (4*S*,5'*S*)-3'-(3-Fluoro-4-morpholinophenyl)-[4,5'-bioxazolidine]-2,2'-dione (258)



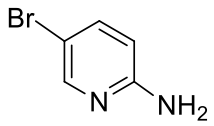
To a solution of oxazolidinone **297** (46 mg, 0.1 mmol) in dichloromethane (1.0 mL), 4N HCl in 1,4-dioxane (0.25 mL, 1.0 mmol) was added and stirred at room temperature overnight. It was concentrated under reduced pressure using a rotary evaporator and used for the next step without purification.

To a suspension of amino salts (0.1 mmol) in anhydrous THF (1.0 mL), *N*,*N*-diisopropylethylamine (33 mg, 0.25 mmol) was added till the salts

were completely dissolved into the organic solvent. Then, triphosgene (30 mg, 0.1 mmol) in anhydrous THF was added at 0 °C over 1 hour and stirred at 0 °C for 2-3 hours. After that, it was warmed to room temperature and stirred overnight. The reaction mixture was washed with distilled water, extracted with ethyl acetate, dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to yield **258** (23 mg, 0.066 mmol, 66% over two steps) as white solid.

TLC (Ethyl acetate) R_f = 0.18 (UV). **IR** (ATR) $\tilde{\nu}$ = 3303 (w), 2969 (w), 2946 (w), 2899 (w), 2868 (w), 2818 (w), 1767 (w), 1735 (m), 1628 (w), 1571 (w), 1469 (w), 1449 (w), 1416 (w), 1389 (w),1352 (w), 1329 (w), 1310 (w), 1272 (w), 1257 (m), 1226 (w), 1170 (w), 1140 (w), 1120 (w), 1098 (w), 1072 (w), 1055 (w), 1022 (w), 937 (w), 924 (w), 879 (w), 851 (w), 817 (w), 751 (w), 728 (w), 658 (w), 637 (w), 607 (w), 582 (w), 526 (w), 506 (w), 472 (w), 445 (w), 413 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 258 (3.21), 204 (3.10) nm. **^{1}H NMR** (500 MHz, DMSO-D_6): δ = 8.17 (s, 1H, NH), 7.50 (dd, $^4J_{H,H}$ = 2.6 Hz, $^3J_{H,F}$ = 15.0 Hz, 1H, CH), 7.33-7.16 (m, 1H, CH), 7.13-6-98 (m, 1H, CH), 4.73 (ddd, $^3J_{H,H}$ = 9.6, 6.2, 3.6 Hz, 1H, CH), 4.44 (t, $^3J_{H,H}$ = 9.1 Hz, 1H, CH), 4.20 (dd, $^3J_{H,H}$ = 8.9, 4.7 Hz, 1H, CH_2), 4.15-4.03 (m, 2H, CH_2), 3,79 (dd, $^3J_{H,H}$ = 9.3, 6.2 Hz, 1H, CH_2), 3.76-3.72 (m, 4H, 2 x CH_2), 3.03-2.93 (m, 4H, 2 x CH_2) ppm. **^{13}C NMR** (125 MHz, DMSO-D_6): δ = 156.4 (d, $^1J_{C,F}$ = 627.9 Hz, C), 155.5 (C), 153.6 (C), 135.6 (d, $^3J_{C,F}$ = 8.8 Hz, C), 133.7 (d, $^2J_{C,F}$ = 10.6 Hz, C), 119.2 (d, $^4J_{C,F}$ = 4.1 Hz, CH), 114.2 (d, $^4J_{C,F}$ = 3.1 Hz, CH), 106.7 (d, $^3J_{C,F}$ = 26.2 Hz, CH), 72.7 (CH), 66.1 (2 x CH_2), 65.4 (CH_2), 53.8 (CH), 50.7 (d, $^4J_{C,F}$ = 2.8 Hz, 2 x CH_2), 46.5 (CH_2) ppm. **^{19}F NMR** (500 MHz, DMSO-D_6): δ = -121.4 ppm. **Optical rotation**: $[\alpha]_D^{20}$ = +5.5 (*c* 0.1, ethanol). **HRMS-ESI**: calcd. for $C_{16}H_{19}FN_3O_5^+$ 352.1303; found 352.1303.

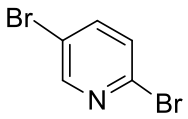
4.2.59 5-Bromopyridin-2-amine (307)



To a solution of 2-aminopyridine (56.4 g, 0.6 mol) in ethanol (600 mL), bromide (34.8 mL, 0.66 mol) was added dropwise at 0 °C and stirred at low temperature for 1hour. The solution was concentrated and a solution of sodium hydroxide (30 g, 0.78 mol) in distilled water (500 mL) was added at 0 °C. Then, it was filtered, the solid was washed with cold water (60 mL) and boiling heptane (2 x 120 mL) to remove 3,5-dibromopyridin-2-amine and the rest was purified by flash column chromatography on silica gel to afford 5-bromopyridin-2-amine **307** (52.46 g, 0.3 mol, 51%) as yellow brown solid.

TLC (cyclohexane/ethyl acetate = 3:1) R_f = 0.34 (UV). **IR** (ATR) $\tilde{\nu}$ = 3452 (w), 3281 (w), 1624 (w), 1586 (w), 1562 (w), 1549 (w), 1512 (w), 1473 (w), 1386 (w), 1310 (w), 1287 (w), 1260 (w), 1140 (w), 1088 (w), 999 (w), 961 (w), 927 (w), 820 (m), 745 (w), 667 (w), 642 (w), 630 (w), 512 (m), 441 (w), 411 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 313 (3.04), 243 (3.69) nm. **MS** (EI, 70 eV): *m/z* (%) = 174 (96) $[M+H]^+$, 173 (7), 172 (100), 156 (6), 147 (47), 145 (31), 135 (6), 119 (7), 93 (9), 91 (8), 79 (14), 73 (24), 66 (20), 51 (6), 44 (34), 41 (8), 40 (12), 39 (11), 38 (5). **GC** (HP-5MS): *I* = 1302. **^{1}H NMR** (500 MHz, MeOD): δ = 9.47 (d, $^4J_{H,H}$ = 2.4 Hz, 1H, CH), 9.08 (dd, $^4J_{H,H}$ = 2.5 Hz, $^3J_{H,H}$ = 8.9 Hz, 1H, CH), 8.08 (d, $^3J_{H,H}$ = 8.9 Hz, 1H, CH), 6.20 (s, 2H, NH_2) ppm. **^{13}C NMR** (125 MHz, MeOD): δ = 159.7 (C), 148.5 (CH), 141.5 (CH), 111.8 (CH), 107.4 (C) ppm.

4.2.60 2,5-Dibromopyridine (308)

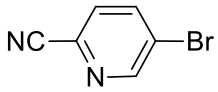


To a cold aqueous 48% hydrogen bromide solution (37 mL, 0.33 mol), 5-bromopyridin-2-amine (13.0 g, 75.2 mmol) was added over 10 minutes. After that, bromide (11 mL, 0.21 mol) was put into the reaction mixture at 0 °C. Then, a solution of sodium nitrite (16.1 g, 0.19 mol) in distilled water (19 mL) was added dropwise at 0 °C and stirred for 30 minutes, subsequently another solution of sodium hydroxide (28.0 g, 0.7 mol) in distilled water (30 mL) was added below room temperature and extracted with ethyl acetate. The collected organic phases were dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator, filtered and purified over column chromatography to afford 2,5-dibromopyridine **308** (8.43 g, 35.6 mmol, 47%) as white crystals.

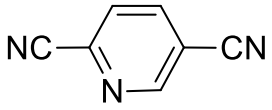
TLC (cyclohexane/ethyl acetate = 20:1) R_f = 0.26 (UV). **IR** (ATR) $\tilde{\nu}$ = 3204 (w), 3099 (w), 1545 (m), 1505 (w), 1470 (w), 1435 (m), 1393 (w), 1355 (w), 1276 (w), 1261 (w), 1158 (w), 1139 (w), 1113 (m), 1089 (m), 1076 (m), 996 (m), 913 (w), 829 (m), 734 (w), 715 (w), 624 (w), 481 (w), 421 (w), 406 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (lg ε) 277 (3.13), 228 (3.70) nm. **MS** (EI, 70 eV): *m/z* (%) = 238.8 (52) $[M+2H]^+$, 237.8 (6), 236.8 (100), 234.8 (54), 158.8 (7), 157.8 (83), 156.8 (7), 155.8 (84), 130.8 (5), 128.8 (7), 76.8 (10), 75.8 (36), 74.8 (8), 50.8 (6), 49.8 (16). **GC** (HP-5MS): *I* = 1261. **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 8.43 (dd, $^5J_{H,H}$ = 0.7 Hz, $^4J_{H,H}$ = 2.5 Hz, 1H, CH), 7.65 (dd, $^4J_{H,H}$ = 2.6 Hz, $^3J_{H,H}$ = 8.4 Hz, 1H, CH), 7.37 (dd, $^5J_{H,H}$ = 0.7 Hz, $^3J_{H,H}$ = 8.4 Hz, 1H, CH) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): δ = 151.3 (CH), 141.1 (CH), 140.5 (C), 130.0 (CH), 120.0 (C) ppm. **HRMS-ESI**: calcd. for $C_5H_3BrN_2^+$ 234.8632; found 234.8633.

4.2.61 5-Bromopicolinonitrile (309)

To a solution of 2,5-dibromopyridine **308** (8.29 g, 35 mmol) in dimethylformamide (80 mL), copper cyanide (3.47 g, 39 mmol) and sodium cyanide (1.93 g, 39 mmol) were added and stirred at 150 °C for 7 hours. After cooling to room temperature, it was poured into distilled water, extracted with ethyl acetate. The collected organic phases were washed with brine, dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator, filtered and purified by flash column chromatography on silica gel to afford 5-bromopicolinonitrile **309** (3.67 g, 20.1 mmol, 57%) as yellow solid and side product pyridine-2,5-dicarbonitrile (0.39 g, 3.0 mmol, 17%) as yellow solid.



TLC (cyclohexane/ethyl acetate = 3:1) R_f = 0.31 (UV). **IR** (ATR) $\tilde{\nu}$ = 3107 (w), 3075 (w), 3050 (w), 2991 (w), 2905 (w), 2233 (w), 1561 (w), 1551 (w), 1481 (w), 1451 (w), 1357 (w), 1282 (w), 1248 (w), 1195 (w), 1125 (w), 1087 (w), 1006 (w), 936 (w), 843 (m), 796 (w), 781 (w), 740 (w), 646 (w), 559 (w), 541 (m), 427 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 275 (3.26), 239 (3.72), 202 (3.71) nm. **MS** (EI, 70 eV): *m/z* (%) = 185 (7) $[M+2H]^+$, 183 (100), 181 (92), 105 (4), 103 (85), 77 (28), 75 (13), 52 (9), 50 (9), 48 (3), 39 (2). **GC** (HP-5MS): *I* = 1291. **^{1}H NMR (**500 MHz, $CDCl_3$): δ = 8.78 (dd, $^5J_{H,H}$ = 0.8 Hz, $^4J_{H,H}$ = 2.3 Hz, 1H, CH), 8.00 (dd, $^4J_{H,H}$ = 2.3 Hz, $^3J_{H,H}$ = 8.3 Hz, 1H, CH), 7.60 (dd, $^5J_{H,H}$ = 0.8 Hz, $^3J_{H,H}$ = 8.3 Hz, 1H, CH) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): δ = 152.7 (CH), 139.9 (CH), 132.2 (C), 129.4 (CH), 125.2 (C), 116.7 (CN) ppm. **HRMS-ESI**: calcd. for $C_6H_3BrN_2^+$ 181.9480; found 181.9480.

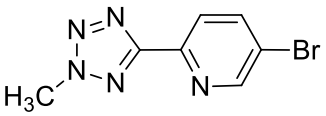


TLC (cyclohexane/ethyl acetate = 3:1) R_f = 0.12 (UV). **IR** (ATR) $\tilde{\nu}$ = 3092 (w), 3069 (w), 3029 (w), 2948 (w), 2241 (w), 1505 (w), 1582 (w), 1556 (w), 1542 (w), 1486 (w), 1462 (m), 1372 (w), 1292 (w), 1250 (w), 1213 (w), 1178 (w), 1129 (w), 1021 (m), 947 (w), 856 (m), 825 (w), 751 (w), 664 (w), 640 (w), 565 (m), 519 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 273 (3.09), 230 (3.60) nm. **MS** (EI, 70 eV): *m/z* (%) = 132 (12) $[M+2H]^+$, 129 (100), 102 (39), 100 (9), 78 (2), 76 (22), 74 (2), 52 (15), 50 (4). **GC** (HP-5MS): *I* = 1288. **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 8.98 (dd, $^5J_{H,H}$ = 0.9 Hz, $^4J_{H,H}$ = 2.1 Hz, 1H, CH), 8.17 (dd, $^4J_{H,H}$ = 2.1 Hz, $^3J_{H,H}$ = 8.1 Hz, 1H, CH), 7.86 (dd, $^5J_{H,H}$ = 1.0 Hz, $^3J_{H,H}$ = 8.1 Hz, 1H, CH) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): δ = 153.3 (CH), 140.8 (CH), 136.7 (C), 128.3 (CH), 115.9 (C), 115.1 (CN), 113.2 (CN) ppm. **HRMS-ESI**: calcd. for $C_7H_3N_3^+$ 129.0327; found 129.0328.

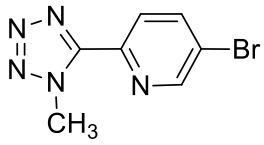
4.2.62 5-Bromo-2-(2-methyl-2*H*-tetrazol-5-yl)pyridine (311) and 5-bromo-2-(1-methyl-1*H*-tetrazol-5-yl)pyridine (312)

To a solution of 5-bromopicolinonitrile **309** (0.37 g, 2.0 mmol) in anhydrous dimethylformamide (4.0 mL), sodium azide (0.16 g, 2.4 mmol) and ammonium chloride (0.11 g, 2.0 mmol) was added and heated at 120 °C for 1 hour. Then, it was diluted with ethyl acetate (20.0 mL) and cooled to room temperature. It was filtered, washed with ethyl acetate, fried in vacuo to obtain white solid. This crude product was used for next step without purification. A mixture of 5-bromo-2-(1*H*-tetrazol-5-yl)pyridine **310** (2.0 mmol) and sodium hydroxide (0.36 g, 9.0 mmol) in anhydrous dimethylformamide (4.0 mL) were stirred at room temperature for 3 hours. It was concentrated under reduced pressure using a rotary

evaporator and re-dissolved in anhydrous dimethylformamide (3.0 mL). Methyl iodide (0.21 mL, 3.4 mmol) was added dropwise at 0 °C and stirred at room temperature for 2 hours. It was then partitioned between water and ethyl acetate. The organic phases were washed with distilled water, dried $MgSO_4$, concentrated under reduced pressure using a rotary evaporator, filtered and purified by flash column chromatography to generate 5-bromo-2-(2-methyl-2*H*-tetrazol-5-yl)pyridine **311** (125 mg, 0.52 mmol, 17%) as white solid and byproduct 5-bromo-2-(1-methyl-1*H*-tetrazol-5-yl)pyridine **312** (120 mg, 0.5 mmol, 17%) as yellow solid.

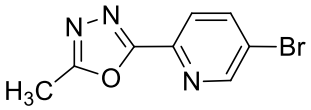


TLC (cyclohexane/ethyl acetate = 3:1) R_f = 0.18 (UV). **IR** (ATR) $\tilde{\nu}$ = 3087 (w), 3046 (w), 2954 (w), 2345 (w), 2109 (w), 1988 (w), 1738 (w), 1579 (w), 1557 (w), 1513 (w), 1443 (w), 1415 (w), 1400 (w), 1377 (w), 1363 (w), 1289 (w), 1235 (w), 1210 (w), 1152 (w), 1131 (w), 1087 (w), 1050 (w), 1016 (w), 1001 (w), 936 (w), 914 (w), 857 (w), 771 (w), 760 (w), 728 (w), 702 (w), 684 (w), 629 (w), 512 (w), 496 (w), 441 (w), 409 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 283 (3.30), 245 (3.63), 201 (3.70) nm. **MS** (EI, 70 eV): *m/z* (%) = 240 (15) $[M]^+$, 239 (7), 214 (7), 213 (96), 212 (34), 211 (100), 210 (35), 208 (19), 207 (66), 191 (5), 185 (35), 184 (19), 183 (34), 182 (17), 158 (29), 156 (31), 135 (5), 133 (9), 105 (6), 104 (10), 103 (28), 77 (12), 76 (37), 75 (7), 62 (5), 51 (9), 50 (9), 43 (33), 40 (19), 38 (5). **GC** (HP-5MS): *I* = 1871. **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 8.80 (d, $^4J_{H,H}$ = 2.3 Hz, 1H, CH), 8.11 (dd, $^5J_{H,H}$ = 0.8 Hz, $^3J_{H,H}$ = 8.4 Hz, 1H, CH), 7.97 (dd, $^4J_{H,H}$ = 2.3 Hz, $^3J_{H,H}$ = 8.4 Hz, 1H, CH), 4.43 (s, 3H, CH_3) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): δ = 164.3 (C), 151.5 (CH), 145.2 (C), 140.0 (CH), 123.5 (CH), 122.3 (C), 40.0 (CH_3) ppm. **HRMS-ESI**: calcd. for $C_7H_6BrN_5^+$ 238.9807; found 238.9806.



TLC (cyclohexane/ethyl acetate = 3:1) R_f = 0.37 (UV). **IR** (ATR) $\tilde{\nu}$ = 3154 (w), 3092 (w), 3051 (w), 3031 (w), 2957 (w), 2921 (w), 2851 (w), 2335 (w), 2080 (w), 1973 (w), 1843 (w), 1740 (w), 1716 (w), 1577 (w), 1556 (w), 1532 (w), 1470 (w), 1453 (w), 1434 (w), 1421 (w), 1391 (w), 1355 (w), 1285 (w), 1272 (w), 1253 (w), 1233 (w), 1212 (w), 1125 (w), 1092 (w), 1062 (w), 1042 (w), 1006 (w), 993 (w), 926 (w), 857 (w), 763 (w), 725 (w), 697 (w), 628 (w), 510 (w), 450 (w), 412 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (lg ε) 246 (3.00), 201 (3.41) nm. **MS** (EI, 70 eV): *m/z* (%) = 241 (29) $[M+H]^+$, 239 (30), 207 (6), 199 (8), 197 (8), 186 (14), 185 (99), 184 (27), 183 (100), 182 (6), 181 (6), 171 (8), 169 (8), 159 (15), 158 (31), 157 (16), 156 (31), 135 (5), 119 (5), 117 (6), 105 (7), 103 (15), 90 (10), 78 (13), 77 (14), 76 (29), 75 (6), 63 (6), 62 (6), 51 (10), 50 (10), 43 (17). **GC** (HP-5MS): *I* = 1912. **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 8.78 (dd, $^5J_{H,H}$ = 0.8 Hz, $^4J_{H,H}$ = 2.2 Hz, 1H, CH), 8.25 (dd, $^5J_{H,H}$ = 0.8 Hz, $^3J_{H,H}$ = 8.5 Hz, 1H, CH), 8.04 (dd, $^4J_{H,H}$ = 2.3 Hz, $^3J_{H,H}$ = 8.5 Hz, 1H, CH), 4.47 (s, 3H, CH_3) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): δ = 151.6 (C), 150.8(CH), 143.4 (C), 140.4 (CH), 125.6 (CH), 123.1 (C), 37.2 (CH_3) ppm. **HRMS-ESI**: calcd. for $C_7H_6BrN_5^+$ 238.9807; found 238.9807.

4.2.63 2-(5-Bromopyridin-2-yl)-5-methyl-1,3,4-oxadiazole (313)

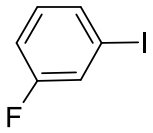


To a solution of 5-bromopicolinonitrile **309** (0.37 g, 2.0 mmol) in anhydrous dimethylformamide (8.0 mL), sodium azide (0.16 g, 2.4 mmol) and ammonium chloride (0.11 g, 2.0 mmol) was added and heated at

120 °C for 1 hour. Then, it was diluted with ethyl acetate (20.0 mL) and cooled to room temperature. It was filtered, washed with ethyl acetate, concentrated under reduced pressure using a rotary evaporator to obtain white solid. This crude product was used for next step without purification. A mixture of 5-bromo-2-(1*H*-tetrazol-5-yl)pyridine **310** (2.0 mmol), fresh distilled acetic anhydride (6.80 mL) and pyridine (0.8 mL, 9.7 mmol) was heated to 150 °C and maintained this temperature for 3 hours. It was cooled to room temperature, poured into distilled water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to produce 2-(5-bromopyridin-2-yl)-5-methyl-1,3,4-oxadiazole **313** (0.17 g, 0.69 mmol) as brown solid.

TLC (cyclohexane/ethyl acetate = 1:1) R_f = 0.45 (UV). **IR** (ATR) $\tilde{\nu}$ = 3088 (w), 3051 (w), 2924 (w), 2853 (w), 2324 (w), 2104 (w), 1991 (w), 1664 (w), 1578 (w), 1477 (w), 1456 (w), 1441 (w), 1401 (w), 1381 (w), 1286 (w), 1257 (w), 1236 (w), 1125 (w), 1099 (w), 1085 (w), 1034 (w), 1003 (w), 972 (w), 958 (w), 944 (w), 841 (w), 758 (w), 748 (w), 703 (w), 688 (w), 666 (w), 628 (w), 505 (w), 491 (w), 442 (w), 408 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (lOg ε) 285 (3.35), 254 (3.49), 201 (3.51) nm. **MS** (EI, 70 eV): *m/z* (%) = 224 (7) $[M+2H]^+$, 221 (100), 128 (3), 111 (3), 97 (12), 94 (51), 75 (19), 70 (4), 68 (3), 63 (2), 51 (6), 39 (1). **GC** (HP-5MS): *I* = 1771. **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 8.80 (d, $^4J_{H,H}$ = 2.3 Hz, 1H, CH), 8.25 (dd, $^3J_{H,H}$ = 8.4 Hz, 1H, CH), 8.04 (dd, $^4J_{H,H}$ = 2.2 Hz, $^3J_{H,H}$ = 8.4 Hz, 1H, CH), 2.65 (s, 3H, CH_3) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): δ = 165.1 (C), 163.7 (C), 151.5 (CH), 142.0 (C), 140.2 (CH), 124.0 (CH), 123.5 (C), 11.3 (CH_3) ppm. **HRMS-ESI**: calcd. for $C_8H_6BrN_3O^+$ 238.9694; found 238.9698.

4.2.64 1-Fluoro-3-iodobenzene (301)

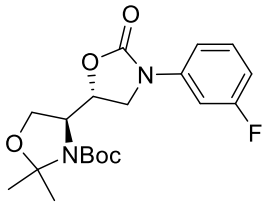


To a solution of 3-fluoroaniline (3.33 g, 30.0 mmol) in 95-97% sulfuric acid (10.9 mL, 19.13 g, 195 mmol) in distilled water (49.0 mL), a solution of sodium nitrite (2.13 g, 30.90 mmol) in distilled water (6.7 mL) was added slowly at 0 °C and stirred at this temperature for one hour. After that, another solution of potassium iodide (7.47 g, 45.0 mmol) in distilled water (10.0 mL) was also put into the reaction mixture gently at 0 °C and maintained this temperature for another hour. The aqueous phase was extracted with ethyl acetate. The collected organic layers were washed with distilled water, 10% sodium bicarbonate aqueous solution (3 x 66.6 mL), dried over $MgSO_4$, filtered, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to afford 1-fluoro-3-iodobenzene **301** (1.63 g, 7.34 mmol, 24%) as purple red oil.

TLC (cyclohexane) R_f = 0.72 (UV). **IR** (ATR) $\tilde{\nu}$ = 3065 (w), 1736 (w), 1582 (s), 1568 (m), 1516 (w), 1466 (s), 1420 (m), 1372 (w), 1328 (w), 1291 (w), 1262 (m), 1241 (w), 1210 (s), 1157 (w), 1078 (w), 1049 (m), 998 (m), 888 (w), 858 (m), 843 (s), 771 (s), 671 (s), 652 (m), 518 (m), 436 (m) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (lOg ε) 267 (3.53), 230 (4.40), 202 (4.53) nm. **MS** (EI, 70 eV): *m/z* (%) = 242 (9) $[M+2H]^+$, 222 (100), 126 (3), 111 (4), 97 (17), 95 (58), 92 (2), 75 (19), 73 (5), 71 (2), 68 (7), 63 (1), 61 (2), 49 (6). **GC** (HP-5MS): *I* = 1027. **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 7.49 (dt, $^4J_{H,H}$ = 1.6 Hz, $^3J_{H,H}$ = 7.2 Hz, 1H, CH), 7.45 (dt, $^4J_{H,H}$ = 1.9 Hz, $^3J_{H,H}$ = 8.1 Hz, 1H, CH), 7.10-7.02 (m, 2H, 2 x CH) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): δ = 162.5 (d, $^1J_{C,F}$ = 251.3 Hz, C), 133.5 (d, $^4J_{C,F}$ = 3.3 Hz,

CH), 131.4 (d, $^{3}J_{C,F}$ = 8.1 Hz, CH), 125.0 (d, $^{2}J_{C,F}$ = 23.5 Hz, CH), 115.1 (d, $^{2}J_{C,F}$ = 20.9 Hz, CH), 93.7 (d, $^{3}J_{C,F}$ = 7.9 Hz, C) ppm. **^{19}F NMR** (500 MHz, $CDCl_3$): δ = -110.49, -111.50 ppm.

4.2.65 (4*S*,5'*R*)-*tert*-Butyl 3'-(3-fluorophenyl)-2,2-dimethyl-2'-oxo-[4,5'-bioxazolidine]-3-carboxylate (304)

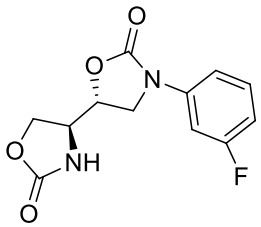


To a solution of bioxazolidine **278** (286 mg, 1.0 mmol) in 1,4-dioxane (2.00 mL), 1-fluoro-3-iodobenzene **301** (222 mg, 1.0 mmol), caesium carbonate (652 mg, 2.0 mmol) and copper iodide (10 mg, 0.05 mmol) were added, evacuated and backfilled with argon three times. After that, *N,N*'-dimethylethylenediamine (10 mg, 0.1 mmol) was put into the reaction and stirred under argon at 110 °C for 4 hours. It was cooled to room temperature, diluted with ethyl acetate, filtered through Celite®, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography to generate **304** (388 mg, 0.98 mmol, 98%, *dr* 55:45) as white solid.

TLC (cyclohexane/ethyl acetate = 3:1) R_f = 0.24 (UV or Ninhydrin Stain). **UV-Vis** (EtOH) λ_{max} (lg ε) 270 (2.27), 238 (3.15), 203 (3.24) nm. **IR** (ATR) $\tilde{\nu}$ = 2980 (w), 2934 (w), 2873 (w), 1747 (m), 1700 (m), 1615 (w), 1589 (w), 1497 (w), 1458 (w), 1412 (m), 1388 (w), 1365 (m), 1351 (w), 1330 (w), 1299 (w), 1281 (w), 1256 (w), 1226 (w), 1199 (m), 1155 (w), 1132 (w), 1113 (w), 1080 (m), 1046 (w), 1026 (w), 998 (w), 946 (w), 877 (w), 851 (m), 808 (w), 769 (m), 751 (m), 679 (w), 667 (w), 616 (w), 57o (w), 518 (w), 456 (w), 417 (w) cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 7.43 (t, $^{3}J_{H,H}$ = 8.7 Hz, 1H, CH), 7.32 (p, $^{3}J_{H,H}$ = 7.7 Hz, 1H, CH), 7.23 (t, $^{4}J_{H,H}$ =

6.4 Hz, 1H, CH), 6.84 (t, $^{3}J_{H,H}$ = 8.8 Hz, 1H, CH), 5.12-4.79 (m, 1H, CH), 4.44-4.24 (m, 1H, CH_2), 4.23-3.98 (m, 3H, CH, CH_2), 3.94 (q, $^{3}J_{H,H}$ = 8.3 Hz, 1H, CH_2), 1.68-1.40 (m, 15H, 5 x CH_3) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): δ = **A**: 163.2 (d, $^{1}J_{C,F}$ = 245.1 Hz, C), 154.3 (C), 153.3 (C), 139.9-139.6 (m, CH), 138.4 (t, $^{4}J_{C,F}$ = 10.5 Hz, CH), 113.6 (CH), 110.9 (d, $^{3}J_{C,F}$ = 21.7 Hz, CH), 106.1 (C), 95.1 (C), 81.5 (C), 71.4 (CH), 63.5 (CH_2), 57.9 (CH), 46.3 (CH_2), 28.4 (CH_3), 27.2 (CH_3), 23.9 (CH_3) ppm.; **B**: 163.2 (d, $^{1}J_{C,F}$ = 245.1 Hz, C), 154.1 (C), 152.0 (C), 139.9-139.6 (m, C), 138.4 (t, $^{4}J_{C,F}$ = 10.5 Hz, CH), 113.6 (CH), 111.1 (d, $^{3}J_{C,F}$ = 21.9 Hz, CH), 105.9 (C), 94.8 (C), 81.3 (C), 71.4 (CH), 63.3 (CH_2), 57.5 (CH), 46.2 (CH_2), 28.5 (CH_3), 26.9 (CH_3), 22.5 (CH_3) ppm. **^{19}F NMR** (500 MHz, $CDCl_3$): δ = -110.8, -111.1 ppm. **Optical rotation**: $[\alpha]_D^{20}$ = -26.5 (*c* 0.2, ethanol). **HRMS-ESI:** calcd. for $C_{19}H_{25}FN_2NaO_5^+$ 403.1640; found 403.1640 [M + Na]$^+$.

4.2.66 (4*S*,5'*R*)-3'-(3-Fluorophenyl)-[4,5'-bioxazolidine]-2,2'-dione (306)



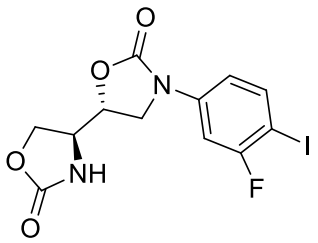
To a solution of oxazolidinone **304** (1.93 g, 5.08 mmol) in dichloromethane (50.8 mL), 4N HCl in 1,4-dioxane (12.7 mL, 50.8 mmol) was added and stirred at room temperature overnight. It was concentrated under reduced pressure using a rotary evaporator and used for next step without purification.

To a solution of amino salts **305** (5.08 mmol) in anhydrous THF (50.8 mL), *N*,*N*-diisopropylethylamine (1.65 g, 12.7 mmol) was added till the

salts were completely dissolved in solvent. Subsequently, triphosgene (1.52 g, 5.08 mmol) in anhydrous THF (50.8 mL) was added at 0 °C over 1 hour and stirred at this temperature for 2-3 hours. It was poured into distilled water, extracted with ethyl acetate, dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to afford **306** (1.18 g, 4.43 mmol, 87%) as white solid.

TLC (cyclohexane/ethyl acetate = 1:5) R_f = 0.24 (UV). **UV-Vis** (EtOH) λ_{max} (lg ε) 251 (2.46), 201 (3.02) nm. **IR** (ATR) $\tilde{\nu}$ = 2980 (w), 2934 (w), 2871 (w), 1747 (m), 1700 (m), 1614 (w), 1590 (w), 1498 (w), 1459 (w), 1413 (w), 1387 (m), 1366 (w), 1347 (m), 1329 (w), 1299 (w), 1282 (w), 1256 (w), 1228 (m), 1200 (m), 1156 (m), 1131 (w), 1113 (w), 1080 (m), 1046 (w), 1026 (w), 999 (w), 950 (w), 877 (w), 851 (m), 810 (w), 770 (m), 752 (m), 679 (w), 668 (w), 615 (w), 571 (w), 519 (w), 456 (w), 417 (w) cm^{-1}.**^{1}H NMR** (500 MHz, DMSO-D_6): δ = 8.18 (s, 1H, NH), 7.40 (dt, $^4J_{H,H}$ = 2.3 Hz, $^3J_{H,H}$ = 11.9 Hz, 1H, CH), 7.35 (td, $^3J_{H,H}$ = 8.2, 6.7 Hz, 1H, CH), 7.23 (dd, $^4J_{H,H}$ = 2.1 Hz, $^3J_{H,H}$ = 8.3 Hz, 1H, CH), 6.88 (td, $^4J_{H,H}$ = 2.5 Hz, $^3J_{H,H}$ = 8.5 Hz, 1H, CH), 4.66 (ddd, $^3J_{H,H}$ = 9.5, 6.2, 3.4 Hz, 1H, CH), 4.34 (t, $^3J_{H,H}$ = 9.0 Hz, 1H, CH_2), 4.11 (dd, $^3J_{H,H}$ = 9.0, 4.7 Hz, 1H , CH_2), 4.09-4.00 (m, 2H, CH_2), 3.78 (dd, $^3J_{H,H}$ = 9.4, 6.2 Hz, 1H, CH) ppm. **^{13}C NMR** (125 MHz, DMSO-D_6): δ = 162.3 (d, $^1J_{C,F}$ = 241.8 Hz, C), 159.0 (C), 153.9 (C), 140.0 (d, $^4J_{C,F}$ = 11.0 Hz, C), 130.6 (t, $^4J_{C,F}$ = 9.5 Hz, CH), 113.8 (d, $^4J_{C,F}$ = 2.7 Hz), 110.1 (d, $^3J_{C,F}$ = 21.0 Hz, CH), 105.1 (d, $^3J_{C,F}$ = 27.0 Hz, CH), 72.8 (CH), 65.5 (CH_2), 53.8 (CH), 46.5 (CH_2), ppm. **^{19}F NMR** (500 MHz, $CDCl_3$): δ = -111.8 ppm. **Optical rotation**: $[\alpha]_D^{20}$ = +3.5 (*c* 0.2, ethanol). **HRMS-ESI:** calcd. for $C_{12}H_{11}FN_2NaO_4^+$ 289.0595; found 289.0595 $[M + Na]^+$.

4.2.67 (4*S*,5'*R*)-3'-(3-Fluoro-4-iodophenyl)-[4,5'-bioxazolidine]-2,2'-dione (299)

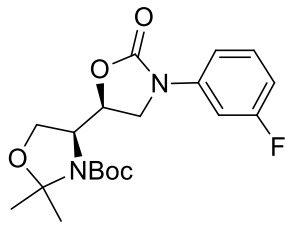


To a solution of bioxazolidinone **306** (184 mg, 0.69 mmol) in trifluoroacetic acid (0.69 mL), *N*-iodosuccinimide (161 mg, 0.72 mmol) was added at room temperature and stirred for 3 hours. Then, it was concentrated under reduced pressure using a rotary evaporator, washed with distilled water, extracted with ethyl acetate, dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and purified over column chromatography on silica gel to obtain **299** (200 mg, 0.51 mmol, 74%) as white solid.

TLC (cyclohexane/ethyl acetate = 1:5) R_f = 0.28 (UV). **IR** (ATR) $\tilde{\nu}$ = 3410 (w), 3098 (w), 2928 (w), 1772 (m), 1744 (m), 1597 (w), 1572 (w), 1478 (m), 1421 (w), 1399 (m), 1333 (m), 1313 (w), 1279 (w), 1224 (m), 1199 (m), 1161 (w), 1138 (w), 1111 (w), 1095 (w), 1070 (w), 1027 (m), 976 (m), 921 (m), 881 (w), 868 (m), 803 (m), 747 (m), 730 (w), 714 (m), 613 (w), 545 (w), 534 (w), 524 (w), 497 (m), 456 (w), 444 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 249 (3.32), 205 (3.30) nm. **^{1}H NMR** (500 MHz, DMSO-D_6): δ = 8.16 (d, $^3J_{H,H}$ = 1.4 Hz, 1H, NH), 7.84 (dd, $^3J_{H,H}$ = 8.7, 7.5 Hz, 1H, CH), 7.54 (dd, $^4J_{H,H}$ = 2.5 Hz, $^3J_{H,H}$ = 10.9 Hz, 1H, CH), 7.20 (dd, $^4J_{H,H}$ = 2.5 Hz, $^3J_{H,H}$ = 8.7 Hz, 1H, CH), 4.75 (ddd, $^4J_{H,H}$ = 6.1 Hz, $^3J_{H,H}$ = 9.5, 3.5 Hz, 1H, CH), 4.45 (t, $^3J_{H,H}$ = 9.1 Hz, 1H, CH), 4.19 (dd, $^3J_{H,H}$ = 9.0, 4.7 Hz, 1H, CH_2), 4.17-4.06 (m, 2H, CH_2), 3.80 (dd, $^4J_{H,H}$ = 6.0 Hz, $^3J_{H,H}$ = 9.4 Hz, 1H, CH_2) ppm. **^{13}C NMR** (125 MHz, DMSO-D_6): δ = 161.2

(d, $^{1}J_{C,F}$ = 240.1 Hz, C), 160.0 (C), 153.8 (C), 140.3 (d, $^{3}J_{C,F}$ = 10.3 Hz, C), 139.1 (d, $^{4}J_{C,F}$ = 2.4 Hz, CH), 115.8 (d, $^{4}J_{C,F}$ = 2.9 Hz, CH), 105.5 (d, $^{2}J_{C,F}$ = 29.8 Hz, CH), 74.2 (d, $^{2}J_{C,F}$ = 26.0 Hz, C), 72.9 (CH), 65.6 (CH_2), 53.9 (CH), 46.4 (CH_2) ppm. **^{19}F NMR** (500 MHz, DMSO-D_6): δ = -93.9 ppm. **Optical rotation**: $[\alpha]_D^{20}$ = +53.7 (*c* 0.4, ethanol). **HRMS-ESI:** calcd. for $C_{12}H_{11}FIN_2NaO_4^+$ 414.9561; found 414.9561 $[M + Na]^+$.

4.2.68 (4*S*,5'*S*)-*tert*-Butyl 3'-(3-fluorophenyl)-2,2-dimethyl-2'-oxo-[4,5'-bioxazolidine]-3-carboxylate (321)

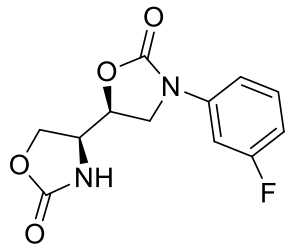


To a solution of bioxazolidine **296** (654 mg, 2.3 mmol) in 1,4-dioxane (5.0 mL), 1-fluoro-3-iodobenzene (508 mg, 2.3 mmol), caesium carbonate (3.0 g, 9.20 mmol) and copper iodide (23 mg, 0.12 mmol) were added, evacuated and backfilled with argon three times. After that, *N,N*'-dimethylethylenediamine (23 mg, 0.23 mmol) was put into the reaction and stirred under argon at 110 °C for 4 hours. It was cooled to room temperature, diluted with ethyl acetate, filtered through Celite®, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography to generate **321** (840 mg, 2.2 mmol, 97%) as white solid.

TLC (cyclohexane/ethyl acetate = 3:1) R_f = 0.29 (UV or Ninhydrin Stain). **UV-Vis** (EtOH) λ_{max} (lg ε) 270 (2.26), 240 (3.05), 203 (3.26) nm. **IR** (ATR) $\tilde{\nu}$ = 2976 (w), 1739 (m), 1679 (w), 1615 (w), 1591 (w), 1496 (w), 1479 (w), 1459 (w), 1410 (w), 1387 (w), 1364 (w), 1349 (w), 1322 (w), 1298 (w), 1280 (w), 1261 (w), 1231 (w), 1217 (w), 1198 (w), 1157 (w),

1120 (w), 1091 (w), 1050 (w), 1032 (w), 1007 (w), 994 (w), 969 (w), 869 (w), 842 (w), 805 (w), 770 (w), 756 (w), 744 (w), 721 (w), 677 (w), 663 (w), 603 (w), 552 (w), 524 (w), 486 (w), 453 (w), 426 (w) cm^{-1}. **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 7.42 (d, $^{3}J_{H,H}$ = 11.0 Hz, 1H, CH), 7.31 (q, $^{3}J_{H,H}$ = 7.2 Hz, 1H, CH), 7.23 (d, $^{3}J_{H,H}$ = 8.9 Hz, 1H, CH), 6.83 (q, $^{3}J_{H,H}$ = 7.9 Hz, 1H, CH), 5.10-4.90 (m, 1H, CH), 4.43-4.24 (m, 1H, CH_2), 4.23-3.97 (m, 3H, CH, CH_2), 3.94 (q, $^{3}J_{H,H}$ = 8.6, 8.2 Hz, 1H, CH_2), 1.72-1.42 (m, 15H, 5 x CH_3) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): δ = **A**: 163.2 (d, $^{1}J_{C,F}$ = 245.2 Hz, C), 154.3 (C), 153.3 (C), 139.7 (s, CH), 130.8 (CH), 113.5 (CH), 112.4-110.3 (m, CH), 106.1 (C), 95.1 (C), 81.5 (C), 71.5 (CH), 63.5 (CH_2), 57.9 (CH), 46.3 (CH_2), 28.5 (CH_3), 27.2 (CH_3), 23.9 (CH_3) ppm.; **B**: 163.2 (d, $^{1}J_{C,F}$ = 245.1 Hz, C), 154.1 (C), 152.0 (C), 139.7 (C), 130.8 (CH), 113.5 (CH), 12.4-110.3 (m, CH), 106.0 (d, $^{3}J_{C,F}$ = 27.0 Hz, CH), 94.8 (C), 81.3 (C), 71.4 (CH), 63.3 (CH_2), 57.6 (CH), 46.2 (CH_2), 28.4 (CH_3), 26.9 (CH_3), 22.5 (CH_3) ppm. **^{19}F NMR** (500 MHz, $CDCl_3$): δ = -110.8, -111.1 ppm. **Optical rotation**: $[\alpha]_D^{20}$ = -0.5 (*c* 0.2, ethanol). **HRMS-ESI:** calcd. for $C_{19}H_{25}FN_2NaO_5^+$ 403.1640; found 403.1640 $[M + Na]^+$.

4.2.69 (4*S*,5'*S*)-3'-(3-Fluorophenyl)-[4,5'-bioxazolidine]-2,2'-dione (323)



To a solution of oxazolidinone **321** (308 mg, 1.0 mmol) in dichloromethane (10.0 mL), 4N HCl in 1,4-dioxane (2.5 mL, 10.0 mmol) was added and stirred at room temperature overnight. It was

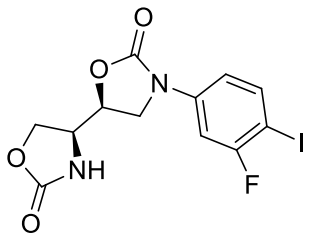
concentrated under reduced pressure using a rotary evaporator and used for next step without purification.

To a solution of amino salts **322** (1.0 mmol) in anhydrous THF (10.0 mL), *N*,*N*-diisopropylethylamine (324 g, 2.5 mmol) was added till the salts were completely dissolved in solvent. Subsequently, triphosgene (300 g, 1.0 mmol) in anhydrous THF (10.0 mL) was added at 0 °C over 1 hour and stirred at this temperature for 2-3 hours. It was poured into distilled water, extracted with ethyl acetate, dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to afford **323** (207 g, 0.78 mmol, 78%) as white solid.

TLC (cyclohexane/ethyl acetate 1:5) R_f = 0.24 (UV). **UV-Vis** (EtOH) λ_{max} (lg ε) 270 (3.03), 236 (3.80), 202 (4.10) nm. **IR** (ATR) $\tilde{\nu}$ = 3467 (w), 3205 (w), 3128 (w), 2921 (w), 1747 (s), 1607 (w), 1589 (w), 1497 (m), 1472 (w), 1454 (w), 1412 (m), 1382 (w), 1330 (w), 1254 (w), 1221 (m), 1199 (m), 1174 (m), 1116 (m), 1075 (w), 1059 (w), 1039 (m), 1020 (m), 996 (w), 963 (w), 934 (m), 897 (w), 871 (m), 858 (m), 796 (m), 741 (m), 720 (w), 683 (m), 664 (m), 620 (w), 554 (w), 520 (w), 463 (w), 428 (w) cm^{-1}. **^{1}H NMR** (500 MHz, DMSO-D_6): δ = 8.13 (s, 1H, NH), 7.45 (dt, $^5J_{H,H}$ = 2.3 Hz, $^3J_{H,H}$ = 11.9 Hz, 1H, CH), 7.39 (td, $^3J_{H,H}$ = 8.3, 6.3 Hz, 1H, CH), 7.28 (dd, $^4J_{H,H}$ = 2.1 Hz, $^3J_{H,H}$ = 8.4 Hz, 1H, CH), 6.92 (td, $^5J_{H,H}$ = 2.6 Hz, $^3J_{H,H}$ = 8.4 Hz, 1H, CH), 4.70 (ddd, $^4J_{H,H}$ = 6.2 Hz, $^3J_{H,H}$ = 9.5, 3.5 Hz, 1H, CH), 4.39 (t, $^3J_{H,H}$ = 9.1 Hz, 1H, CH), 4.15 (dd, $^3J_{H,H}$ = 9.0, 4.7 Hz, 1H, CH_2), 4.12-4.03 (m, 2H, CH_2), 3.77 (dd, $^4J_{H,H}$ = 6.1 Hz, $^3J_{H,H}$ = 9.4 Hz, 1H, CH_2) ppm. **^{13}C NMR** (125 MHz, DMSO-D_6): δ = 162.3 (d, $^1J_{C,F}$ = 241.8 Hz, C), 159.0 (C), 153.9 (C), 140.0 (d, $^3J_{C,F}$ = 11.0 Hz, C), 130.6 (d, $^4J_{C,F}$ = 9.5 Hz, CH), 113.7 (d, $^4J_{C,F}$ = 2.7 Hz, CH), 110.1 (d, $^2J_{C,F}$ = 21.0 Hz, CH), 105.0 (d, $^2J_{C,F}$ = 27.0 Hz, CH), 72.8 (CH), 65.5 (CH_2), 53.9 (CH), 46.5

(CH_2) ppm. **^{19}F NMR** (500 MHz, DMSO-D_6): δ = -111.8 ppm. **Optical rotation**: $[\alpha]^{20}_D$ = 17.1 (*c* 0.3, EtOH). **HRMS-ESI:** calcd. for $C_{12}H_{11}FN_2NaO_4^+$ 289.0595; found 289.0595 $[M + Na]^+$.

4.2.70 (4*S*,5'*S*)-3'-(3-Fluoro-4-iodophenyl)-[4,5'-bioxazolidine]-2,2'-dione (324)

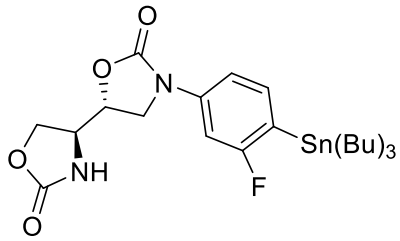


To a solution of bioxazolidinone **323** (203 mg, 0.76 mmol) in trifluoroacetic acid (0.76 mL), *N*-iodosuccinimide (178 mg, 0.8 mmol) was added at room temperature and stirred for 3 hours. Then, it was concentrated under reduced pressure using a rotary evaporator, washed with distilled water, extracted with ethyl acetate, dried over $MgSO_4$, concentrated under reduced pressure using a rotary evaporator and purified by flash column chromatography on silica gel to yield **324** (231 mg, 0.59 mmol, 78%) as white solid.

TLC (cyclohexane/ethyl acetate = 1:5) R_f = 0.28 (UV). **IR** (ATR) $\tilde{\nu}$ = 3408 (w), 3254 (w), 2962 (w), 2918 (w), 1737 (m), 1715 (m), 1601 (w), 1568 (w), 1479 (w), 1454 (w), 1431 (w), 1417 (w), 1398 (w), 1342 (w), 1323 (w), 1292 (w), 1264 (w), 1234 (w), 1205 (w), 1164 (w), 1130 (w), 1115 (w), 1074 (w), 1040 (w), 969 (w), 925 (w), 873 (w), 842 (w), 805 (w), 779 (w), 761 (w), 746 (w), 728 (w), 688 (w), 671 (w), 603 (w), 572 (w), 544 (w), 525 (w), 504 (w), 454 (w) cm^{-1}. **UV-Vis** (EtOH) λ_{max} (log ε) 247 (3.41), 206 (3.07) nm. **^{1}H NMR** (500 MHz, DMSO-D_6): δ = 8.16 (d, $^3J_{H,H}$ = 1.6 Hz, 1H, NH), 7.84 (dd, $^3J_{H,H}$ = 8.7, 7.5 Hz, 1H, CH), 7.54 (dd, $^4J_{H,H}$ = 2.5 Hz, $^3J_{H,H}$ = 10.8 Hz, 1H, CH), 7.20 (dd, $^4J_{H,H}$ = 2.5 Hz, $^3J_{H,H}$ =

8.7 Hz, 1H, CH), 4.75 (ddd, $^{4}J_{H,H}$ = 6.1 Hz, $^{3}J_{H,H}$ = 9.5, 3.5 Hz, 1H, CH), 4.43 (t, $^{3}J_{H,H}$ = 9.1 Hz, 1H, CH), 4.19 (dd, $^{3}J_{H,H}$ = 9.0, 4.7 Hz, 1H, CH_2), 4.17-4.06 (m, 2H, CH_2), 3.80 (dd, $^{4}J_{H,H}$ = 6.0 Hz, $^{3}J_{H,H}$ = 9.4 Hz, 1H, CH_2) ppm. **^{13}C NMR** (125 MHz, DMSO-D_6): δ = 161.2 (d, $^{1}J_{C,F}$ = 240.2 Hz, C), 158.9 (C), 153.7 (C), 140.2 (d, $^{3}J_{C,F}$ = 10.3 Hz, C), 139.0 (d, $^{4}J_{C,F}$ = 3.3 Hz, CH), 115.7 (d, $^{4}J_{C,F}$ = 3.0 Hz, CH), 105.4 (d, $^{2}J_{C,F}$ = 29.8 Hz, CH), 74.2 (d, $^{2}J_{C,F}$ = 26.1 Hz, C), 72.9 (CH), 65.4 (CH_2), 53.8 (CH), 46.3 (CH_2) ppm. **^{19}F NMR** (500 MHz, DMSO-D_6): δ = -93.9 ppm. **Optical rotation**: $[\alpha]_D^{20}$ = +10.0 (*c* 0.1, ethanol). **HRMS-ESI:** calcd. for $C_{12}H_{11}FIN_2NaO_4^+$ 414.9561; found 414.9561 $[M + Na]^+$.

4.2.71 (4*S*,5'*R*)-3'-(3-Fluoro-4-(tributylstannyl)phenyl)-[4,5'-bioxazolidine]-2,2'-dione (319)

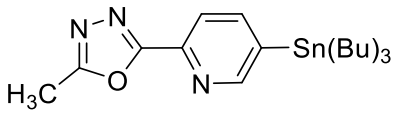


To a solution of iodo compound **299** (24 mg, 0.061 mmol) in 1,4-dioxane (1 mL), hexabutyldistannane (0.02 mL, 23 mg, 0.04 mmol) and bis(triphenylphosphine)palladium(II) dichloride (4 mg, 0.005 mmol) were added and stirred at 100 °C for 2 hours. The solution was filtered through Celite®, concentrated in vacuo and purified by flash column chromatography on silica gel with 10% potassium fluoride to afford **319** (18 mg, 0.035 mmol, 53%) as colourless oil.

TLC (cyclohexane/ethyl acetate = 1:2) R_f = 0.29 (UV). **^{1}H NMR** (500 MHz, C_6D_6): δ = 8.50 (s, 1H, NH), 7.54-7.49 (m, 1H, CH), 7.49-7.42 (m, 2H, 2 x CH), 3.79-3.69 (m, 2H, CH_2), 3.64 (dd, $^{3}J_{H,H}$ = 9.3, 4.7 Hz, 1H, CH), 3.27-3.14 (m, 3H, CH, CH_2), 1.75-1.54 (m, 6H, 3 x CH_2), 1.45-1.35

(m, 6H, 3 x CH_2), 1.33-1.13 (m, 6H, 3 x CH_2), 0.93 (t, $^3J_{H,H}$ = 7.3 Hz, 9H, 3 x CH_3) ppm. **^{13}C NMR** (125 MHz, C_6D_6): δ = 168.3 (d, $^1J_{C,F}$ = 232.9 Hz, C), 160.2 (C), 153.5 (C), 140.9 (d, $^3J_{C,F}$ = 10.8 Hz, C), 138.1 (d, $^4J_{C,F}$ = 1.7 Hz, CH), 121.6 (d, $^3J_{C,F}$ = 46.2 Hz, C), 114.2 (d, $^4J_{C,F}$ = 2.6 Hz, CH), 72.6 (CH), 66.1 (CH_2), 54.1 (CH), 46.1 (CH_2), 29.5 (3 x CH_2), 27.7 (3 x CH_2), 13.9 (3x CH_3), 10.3 (3 x CH_2) ppm. **^{19}F NMR** (500 MHz, C_6D_6): δ = -91.6 ppm. **HRMS-ESI:** calcd. for $C_{24}H_{38}FN_2NaO_4Sn^+$ 579.2756; found 579.1656 $[M + Na]^+$.

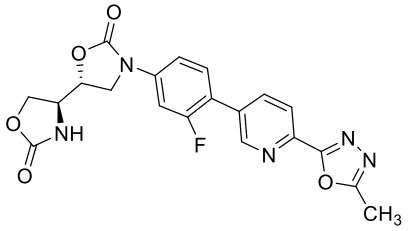
4.2.72 2-Methyl-5-(5-(tributylstannyl)pyridin-2-yl)-1,3,4-oxadiazole (320)



To a solution of brominated pyridine **313** (24 mg, 0.1 mmol) in 1,4-dioxane (1 mL), hexabutyldistannane (0.03 mL, 0.06 mmol) and bis(triphenylphosphine)palladium(II) dichloride (7 mg, 0.009 mmol) were added and stirred at 100 °C for 2 hours. The solution was filtered through Celite®, concentrated in vacuo and purified by flash column chromatography on silica gel with 10% potassium fluoride to afford **320** (32 mg, 0.07 mmol, 70%) as colourless oil.

TLC (cyclohexane/ethyl acetate = 5:1) R_f = 0.2 (UV). **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 8.79-8.62 (m, 1H, CH), 8.14 (dd, $^4J_{H,H}$ = 1.0 Hz, $^3J_{H,H}$ = 7.6 Hz, 1H, CH), 8.02-7.88 (m, 1H, CH), 1.72-1.42 (m, 6H, 3 x CH_2), 1.40-1.26 (m, 6H, 3 x CH_2), 1.22-1.05 (m, 6H, 3 x CH_2), 0.93 (t, $^3J_{H,H}$ = 7.3 Hz, 12H, 4 x CH_3) ppm. **^{13}C NMR** (125 MHz, $CDCl_3$): δ = 164.8 (C), 164.6 (C), 156.2 (CH), 145.6 (CH), 142.8 (C), 141.8 (C), 122.5 (CH), 29.1 (3 x CH_2), 27.4 (3 x CH_2), 13.8 (3x CH_3), 11.3 (CH_3), 10.0 (3 x CH_2) ppm.

4.2.73 (4*S*,5'*R*)-3'-(3-Fluoro-4-(6-(5-methyl-1,3,4-oxadiazol-2-yl)pyridin-3-yl)phenyl)-[4,5'-bioxazolidine]-2,2'-dione (104)

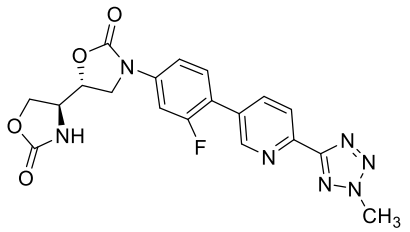


Method A: To a solution of organostannane **319** (36 mg, 0.069 mmol) in *N*-methyl-2-pyrrolidon (1 mL), brominated pyridine **313** (18 mg, 0.076 mmol), lithium chloride (11 mg, 0.23 mmol), bis(triphenylphosphine)palladium(II) dichloride (3 mg, 0.004 mmol) were added and stirred at 120 °C for 4 hours. Then, it was cooled to room temperature, poured into distilled water, extracted three times with ethyl acetate. The collected organic phases were washed with birne, dried over $MgSO_4$, concentrated in vacuo and purified by column chromatography on silica gel with 10% potassium fluoride to achieve **104** (8.8 mg, 0.021 mmol, 30%) as white solid.

Method B: To a solution of organostannane **320** (11 mg, 0.024 mmol) in *N*-methyl-2-pyrrolidon (1 mL), iodo compound **299** (11 mg, 0.027 mmol), lithium chloride (4 mg, 0.08 mmol), bis(triphenylphosphine)palladium(II) dichloride (1 mg, 0.001 mmol) were added and stirred at 120 °C for 4 hours. Then, it was cooled to room temperature, poured into distilled water, extracted three times with ethyl acetate. The collected organic phases were washed with birne, dried over $MgSO_4$, concentrated in vacuo and purified by column chromatography on silica gel with 10% potassium fluoride to achieve **104** (7.6 mg, 0.018 mmol, 26%) as white solid.

TLC (chloroform/methanol = 20:1) R_f = 0.14 (UV). **^{1}H NMR** (500 MHz, DMSO-D_6): δ = 8.92 (q, $^3J_{H,H}$ = 1.6 Hz, 1H, NH), 8.21 (d, $^3J_{H,H}$ = 1.4 Hz, 2H, 2 x CH), 8.14-8-08 (m, 1H, CH), 7.76 (t, $^3J_{H,H}$ = 8.8 Hz, CH), 7.66 (dd, $^4J_{H,H}$ = 2.3 Hz, $^3J_{H,H}$ = 13.6 Hz, 1H, CH), 7.47 (dd, $^4J_{H,H}$ = 2.3 Hz, $^3J_{H,H}$ = 8.6 Hz, 1H, CH), 4.78 (ddd, $^4J_{H,H}$ = 4.7 Hz, $^3J_{H,H}$ = 9.0, 6.3 Hz, 1H, CH), 4.43 (t, $^3J_{H,H}$ = 8.4 Hz, 1H, CH_2), 4.26-4.11 (m, 3H, CH, CH_2), 3.85 (dd, $^3J_{H,H}$ = 9.5, 6.4 Hz, 1H, CH_2), 2.6 (s, 3H, CH_3) ppm. **^{13}C NMR** (125 MHz, DMSO-D_6): δ = 165.0 (C), 163.4 (C), 159.4 (d, $^1J_{C,F}$ = 246.1 Hz, C), 158.8 (C), 153.6 (C), 149.5 (d, $^4J_{C,F}$ = 4.2 Hz, CH), 141.7 (C), 140.5 (d, $^3J_{C,F}$ = 11.3 Hz, C), 137.4 (d, $^4J_{C,F}$ = 3.5 Hz, CH), 132.6 (d, $^5J_{C,F}$ = 2.2 Hz, C), 131.2 (d, $^4J_{C,F}$ = 4.1 Hz, CH), 122.5 (CH), 118.7 (d, $^3J_{C,F}$ = 13.2 Hz, C), 114.3 (d, $^4J_{C,F}$ = 3.0 Hz, CH), 105.7 (d, $^2J_{C,F}$ = 28.4 Hz), 73.0 (CH), 64.8 (CH_2), 53.2 (CH), 45.6 (CH_2), 10.7 (CH_3) ppm. **^{19}F NMR** (500 MHz, DMSO-D_6): δ = -115.8 ppm.

4.2.74 (4*S*,5'*R*)-3'-(3-Fluoro-4-(6-(2-methyl-2*H*-tetrazol-5-yl)pyridin-3-yl)phenyl)-[4,5'-bioxazolidine]-2,2'-dione (102)

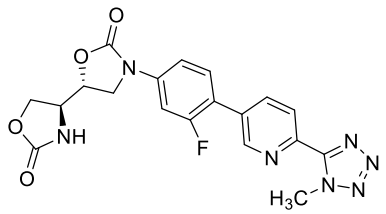


To a solution of organostannane **319** (18 mg, 0.035 mmol) in *N*-methyl-2-pyrrolidon (1 mL), brominated pyridine **311** (9 mg, 0.038 mmol), lithium chloride (6 mg, 0.12 mmol), bis(triphenylphosphine)palladium(II) dichloride (2 mg, 0.002 mmol) were added and stirred at 120 °C for 4 hours. Then, it was cooled to room temperature, poured into distilled water, extracted three times with ethyl acetate. The collected organic phases were washed with birne, dried over $MgSO_4$, concentrated in vacuo and purified by column chromatography on silica gel with 10%

potassium fluoride to achieve **102** (4.7 mg, 0.011 mmol, 32%) as white solid.

TLC (chloroform/methanol = 20:1) R_f = 0.06 (UV). **^{1}H NMR** (500 MHz, DMSO-D_6): δ = 8.91 (s, 1H, NH), 8.22-8.14 (m, 2H, 2 x CH), 8.12 (s, 1H, CH), 7.74 (t, $^3J_{H,H}$ = 8.8 Hz, CH), 7.65 (dd, $^4J_{H,H}$ = 2.2 Hz, $^3J_{H,H}$ = 13.6 Hz, 1H, CH), 7.46 (dd, $^4J_{H,H}$ = 2.3 Hz, $^3J_{H,H}$ = 8.0 Hz, 1H, CH), 4.78 (ddd, $^4J_{H,H}$ = 4.7 Hz, $^3J_{H,H}$ = 8.9, 6.4 Hz, 1H, CH), 4.43 (d, $^3J_{H,H}$ = 8.7 Hz, 1H, CH_2), 4.44 (s, 3H, CH_3), 4.42 (d, $^3J_{H,H}$ = 8.7 Hz, 1H, CH_2), 4.25-4.10 (m, 2H, CH_2), 3.85 (dd, $^3J_{H,H}$ = 9.5, 6.3 Hz, 1H, CH) ppm. **^{13}C NMR** (125 MHz, DMSO-D_6): δ = 163.9 (C), 158.8 (C), 159.3 (d, $^1J_{C,F}$ = 245.8 Hz, C), 158.8 (C), 153.6 (C), 149.5 (d, $^4J_{C,F}$ = 4.0 Hz, CH), 145.2 (C), 140.2 (d, $^3J_{C,F}$ = 11.2 Hz, C), 137.3 (d, $^4J_{C,F}$ = 3.4 Hz, CH), 131.6 (d, $^5J_{C,F}$ = 2.0 Hz), 131.1 (d, $^4J_{C,F}$ = 4.3 Hz, CH), 122.2 (CH), 119.0 (d, $^3J_{C,F}$ = 13.3 Hz, C), 114.3 (d, $^4J_{C,F}$ = 3.0 Hz, CH), 105.7 (d, $^2J_{C,F}$ = 28.4 Hz, CH), 73.0 (CH), 64.8 (CH_2), 53.2 (CH), 45.6 (CH_2) ppm. **^{19}F NMR** (500 MHz, DMSO-D_6): δ = -115.8 ppm.

4.2.75 (4*S*,5'*R*)-3'-(3-Fluoro-4-(6-(1-methyl-1*H*-tetrazol-5-yl)pyridin-3-yl)phenyl)-[4,5'-bioxazolidine]-2,2'-dione (103)



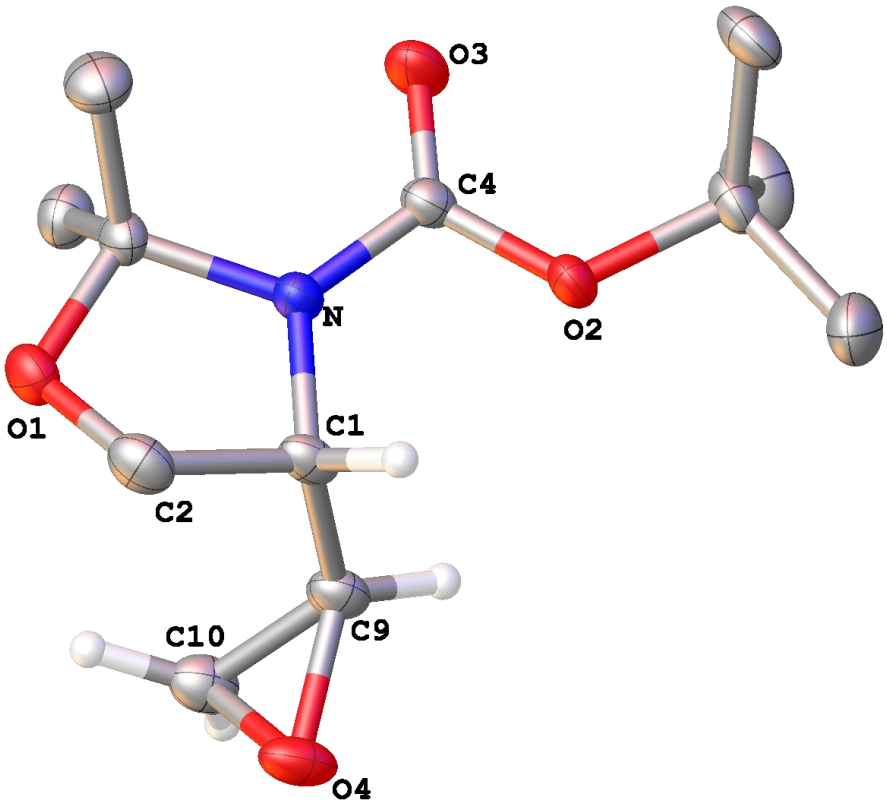
To a solution of organostannane **319** (32 mg, 0.062 mmol) in *N*-methyl-2-pyrrolidon (1 mL), brominated pyridine **312** (16 mg, 0.068 mmol), lithium chloride (10 mg, 0.2 mmol), bis(triphenylphosphine)palladium(II) dichloride (3.5 mg, 0.003 mmol) were added and stirred at 120 °C for 4 hours. Then, it was cooled to room temperature, poured into distilled water, extracted three times with ethyl acetate. The collected organic

phases were washed with birne, dried over $MgSO_4$, concentrated in vacuo and purified by column chromatography on silica gel with 10% potassium fluoride to achieve **103** (9.9 mg, 0.023 mmol, 38%) as white solid.

TLC (chloroform/methanol = 20:1) R_f = 0.08 (UV). **^{1}H NMR** (500 MHz, DMSO-D_6): δ = 9.08-8.98 (m, 1H, NH), 8.35 (dd, $^4J_{H,H}$ = 0.9 Hz, $^3J_{H,H}$ = 8.3 Hz, 2H, CH), 8.29 (ddd, $^4J_{H,H}$ = 1.3 Hz, $^3J_{H,H}$ = 8.2, 2.4 Hz, 1H, CH), 8.20-8.11 (m, 1H, CH), 7.81 (t, $^3J_{H,H}$ = 8.8 Hz, 1H, CH), 7.70 (dd, $^4J_{H,H}$ = 2.3 Hz, $^3J_{H,H}$ = 13.5 Hz, 1H, CH), 7.52 (dd, $^4J_{H,H}$ = 2.3 Hz, $^3J_{H,H}$ = 8.6 Hz, 1H, CH), 4.83 (ddd, $^4J_{H,H}$ = 4.4 Hz, $^3J_{H,H}$ = 9.0, 6.3 Hz, 1H, CH), 4.46 (d, $^3J_{H,H}$ = 4.3 Hz, 1H, CH_2), 4.30-4.13 (m, 3H, CH, CH_2), 3.89 (dd, $^3J_{H,H}$ = 9.4, 6.3 Hz, 1H, CH_2) ppm. **^{13}C NMR** (125 MHz, DMSO-D_6): δ = 159.4 (d, $^1J_{C,F}$ = 246.6 Hz, C), 158.8 (C), 153.6 (C), 151.8 (C), 149.0 (d, $^4J_{C,F}$ = 4.0 Hz, CH), 143.1 (C), 140.5 (d, $^3J_{C,F}$ = 11.3 Hz, C), 137.6 (d, $^4J_{C,F}$ = 3.4 Hz, CH), 132.2 (d, $^5J_{C,F}$ = 2.1 Hz, C), 131.2 (d, $^4J_{C,F}$ = 4.2 Hz, CH), 124.0 (C), 118.7 (d, $^3J_{C,F}$ = 13.3 Hz, CH), 114.3 (d, $^4J_{C,F}$ = 3.0 Hz, CH), 105.7 (d, $^2J_{C,F}$ = 28.4 Hz, CH), 73.0 (CH), 64.8 (CH_2), 53.2 (CH), 45.6 (CH_2), 36.7 (CH_3) ppm. **^{19}F NMR** (500 MHz, DMSO-D_6): δ = -115.9 ppm.

4.3 Crystallographic data

4.3.1 Crystal structure analysis of compound 281



Crystal habitus	clear colourless needle	μ/mm^{-1}	0.728
Device type	Bruker X8-KappaApexII	F(000)	528.0
Empirical formula	$C_{12}H_{21}NO_4$	Crystal size/mm^3	0.24 x 0.2 x 0.1
Moiety formula	$C_{12}H_{21}NO_4$	Absorption correction	empirical
Formula weight	243.30	Tmin; Tmax	0.5263; 0.7536
Temperature/K	100	Radiation	CuKα (λ = 1.54178)
Crystal system	Orthorhombic	2Θ range for data collection/^{o}	10.018 to 144.24^o
Space group	$P2_12_12_1$	Completeness to theta	0.995
a/Å	5.7773(2)	Index ranges	- 4 ≤ h ≤ 7, -9 ≤ h ≤ 11, -28 ≤ h ≤ 30
b/Å	9.4348(3)	Reflections collected	9624
c/Å	25.0285(6)	Independent reflections	2614 [R_{int} = 0.0403, R_{sigma} = 0.0369]
α/^{o}	90	Data/restraints/parameters	2614/0/160
β/^{o}	90	Goodness-of-fit on F^2	1.036
γ/^{o}	90	Final R indexes [I>=2σ (I)]	R_1 = 0.0303, wR_2 = 0.0676
Volume/Å^3	1364.24(7)	Final R indexes [all Data]	R_1 = 0.0350, wR_2 = 0.0697
Z	4	Largest diff. peak/hole/ e Å^{-3}	0.17/-0.12
ρ_{calc}g/cm^3	1.185	Flack parameter	0.16(10)

Table 18 Details of X-ray crystal structure determination of compound **281**.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
O1	C2	1.429(2)	N	C4	1.357(2)
O1	C3	1.434(2)	C1	C2	1.513(3)
O2	C4	1.349(2)	C1	C9	1.514(3)
O2	C5	1.475(2)	C3	C11	1.522(3)
O3	C4	1.214(2)	C3	C12	1.508(3)
O4	C9	1.444(2)	C5	C6	1.514(2)
O4	C10	1.443(2)	C5	C7	1.520(3)
N	C1	1.467(2)	C5	C8	1.515(3)
N	C3	1.485(2)	C9	C10	1.462(3)

Table 19 Details of X-ray crystal structure determination of compound **281**.

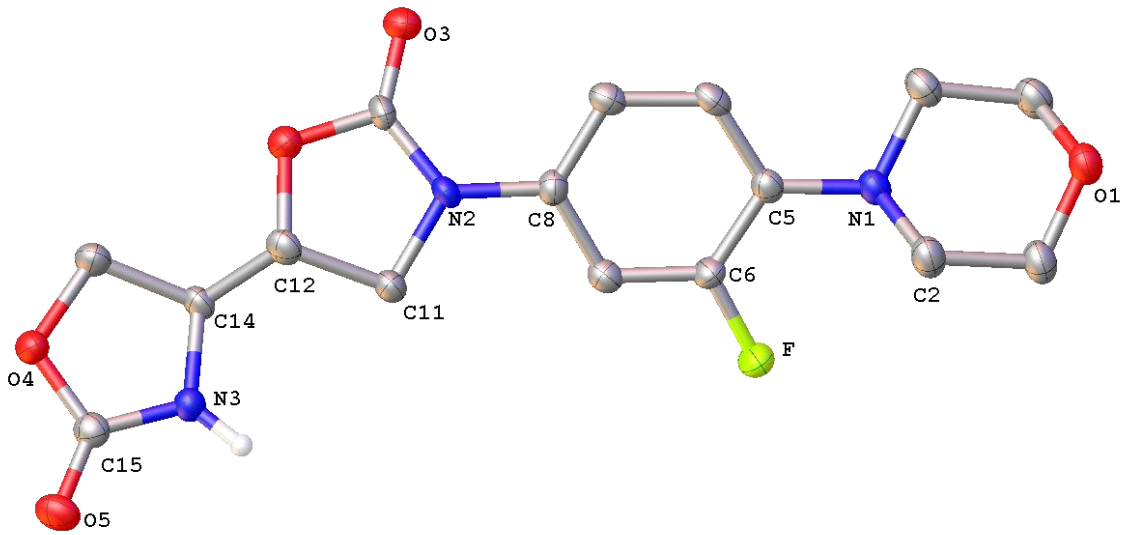
Atom	Atom	Atom	Angle/˚	Atom	Atom	Atom	Angle/˚
C2	O1	C3	108.17(13)	C12	C3	C11	113.02(16)
C4	O2	C5	120.56(14)	O2	C4	N	109.96(15)
C10	O4	C9	60.86(11)	O3	C4	O2	125.77(16)
C1	N	C3	111.45(13)	O3	C4	N	124.26(16)
C4	N	C1	125.56(15)	O2	C5	C6	110.79(15)
C4	N	C3	122.77(14)	O2	C5	C7	101.52(14)
N	C1	C2	99.96(14)	O2	C5	C8	109.94(16)
N	C1	C9	109.73(14)	C6	C5	C7	110.89(17)
C2	C1	C9	115.99(14)	C6	C5	C8	112.64(18)
O1	C2	C1	104.69(14)	C8	C5	C7	110.52(19)
O1	C3	N	102.37(13)	O4	C9	C1	116.30(15)
O1	C3	C11	110.11(15)	O4	C9	C10	59.55(11)
O1	C3	C12	107.45(14)	C10	C9	C1	123.04(17)
N	C3	C11	110.97(14)	O4	C10	C9	59.59(11)
N	C3	C12	112.30(15)				

Table 20 Details of X-ray crystal structure determination of compound **281**.

A	B	C	D	Angle/˚	A	B	C	D	Angle/˚
N	C1	C2	O1	32.75(16)	C3	N	C1	C9	104.28(16)
N	C1	C9	O4	-160.17(15)	C3	N	C4	O2	174.30(15)
N	C1	C9	C10	-90.8(2)	C3	N	C4	O3	-6.3(3)
C1	N	C3	O1	-3.04(18)	C4	O2	C5	C6	59.5(2)
C1	N	C3	C11	114.42(16)	C4	O2	C5	C7	177.30(17)
C1	N	C3	C12	-117.99(16)	C4	O2	C5	C8	-65.7(2)
C1	N	C4	O2	0.1(2)	C4	N	C1	C2	156.65(16)
C1	N	C4	O3	179.51(17)	C4	N	C1	C9	-81.0(2)
C1	C9	C10	O4	-103.34(18)	C4	N	C3	O1	-177.96(15)
C2	O1	C3	N	25.06(17)	C4	N	C3	C11	-60.5(2)
C2	O1	C3	C11	-93.01(17)	C4	N	C3	C12	67.1(2)
C2	O1	C3	C12	143.50(15)	C5	O2	C4	O3	2.4(3)
C2	C1	C9	O4	-47.9(2)	C5	O2	C4	N	-178.26(14)
C2	C1	C9	C10	21.4(2)	C9	C1	C2	O1	-85.09(17)
C3	O1	C2	C1	-37.68(17)	C10	O4	C9	C1	114.51(19)
C3	N	C1	C2	-18.10(17)					

Table 21 Details of X-ray crystal structure determination of compound **281.**

4.3.2 Crystal structure analysis of compound 92



Crystal habitus	clear colourless plank	μ/mm^{-1}	0.124
Device type	Bruker X8-KappaApexII	F(000)	184.0
Empirical formula	$C_{16}H_{18}FN_3O_5$	Crystal size/mm^3	0.1 x 0.02 x 0.02
Moiety formula	$C_{16}H_{18}FN_3O_5$	Absorption correction	Empirical
Formula weight	351.33	Tmin; Tmax	0.6682; 0.7459
Temperature/K	100	Radiation	CuKα (λ = 0.71073)
Crystal system	Triclinic	2Θ range for data collection/^{o}	7.38 to 55.998^o
Space group	P1	Completeness to theta	0.997
a/Å	6.1801(7)	Index ranges	- 8 ≤ h ≤ 8, -8 ≤ h ≤ 8, -14 ≤ h ≤ 14
b/Å	6.2673(7)	Reflections collected	8022
c/Å	11.0167(14)	Independent reflections	3567 [R_{int} = 0.0461, R_{sigma} = 0.0756]
α/^{o}	81.777(7)	Data/restraints/parameters	3567/3/227
β/^{o}	85.382(7)	Goodness-of-fit on F^2	1.058
γ/^{o}	63.329(6)	Final R indexes [I>=2σ (I)]	R_1 = 0.0649, wR_2 = 0.1416
Volume/Å^3	377.32(8)	Final R indexes [all Data]	R_1 = 0.0869, wR_2 = 0.1687
Z	1	Largest diff. peak/hole/ e Å^{-3}	0.29/-0.37
ρ_{calc}g/cm^3	1.546	Flack parameter	-2(2)

Table 22 Details of X-ray crystal structure determination of compound **92**.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
F	C6	1.353(5)	N2	C13	1.355(7)
O1	C1	1.429(7)	N3	C14	1.455(6)
O1	C4	1.424(7)	N3	C15	1.352(6)
O2	C12	1.449(6)	C1	C2	1.515(7)
O2	C13	1.353(6)	C3	C4	1.504(8)
O3	C13	1.227(6)	C5	C6	1.394(7)
O4	C15	1.351(6)	C5	C10	1.397(7)
O4	C16	1.441(6)	C6	C7	1.377(7)
O5	C15	1.215(6)	C7	C8	1.394(7)
N1	C2	1.472(7)	C8	C9	1.395(7)
N1	C3	1.467(6)	C9	C10	1.393(7)
N1	C5	1.424(6)	C11	C12	1.532(7)
N2	C8	1.415(6)	C12	C14	1.516(7)
N2	C11	1.450(6)	C14	C16	1.538(8)

Table 23 Details of X-ray crystal structure determination of compound **92**.

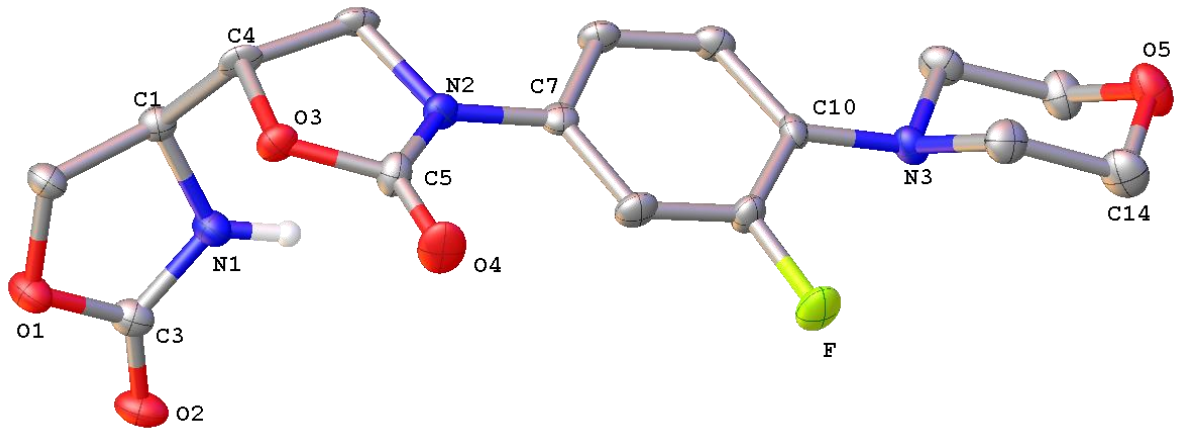
Atom	Atom	Atom	Angle/˚	Atom	Atom	Atom	Angle/˚
C4	O1	C1	109.2(4)	C6	C7	C8	119.1(5)
C13	O2	C12	109.2(4)	C7	C8	N2	118.8(5)
C15	O4	C16	110.3(4)	C7	C8	C9	119.1(5)
C3	N1	C2	109.6(4)	C9	C8	N2	122.1(5)
C5	N1	C2	113.9(4)	C10	C9	C8	119.7(5)
C5	N1	C3	115.0(4)	C9	C10	C5	122.7(4)
C8	N2	C11	123.2(4)	N2	C11	C12	101.4(4)
C13	N2	C8	125.2(4)	O2	C12	C11	103.9(4)
C13	N2	C11	110.9(4)	O2	C12	C14	108.4(4)
C15	N3	C14	111.6(4)	C14	C12	C11	114.6(4)
O1	C1	C2	111.2(5)	O2	C13	N2	110.3(4)
N1	C2	C1	110.4(4)	O3	C13	O2	120.9(5)
N1	C3	C4	109.5(5)	O3	C13	N2	128.8(5)
O1	C4	C3	111.7(4)	N3	C14	C12	109.6(4)
C6	C5	N1	121.1(4)	N3	C14	C16	101.0(4)
C6	C5	C10	115.2(4)	C12	C14	C16	113.4(4)
C10	C5	N1	123.7(4)	O4	C15	N3	109.8(4)
F	C6	C5	118.9(4)	O5	C15	O4	122.3(5)
F	C6	C7	116.9(4)	O5	C15	N3	127.9(5)
C7	C6	C5	124.2(5)	O4	C16	C14	104.6(4)

Table 24 Details of X-ray crystal structure determination of compound **92**.

A	B	C	D	Angle/°	A	B	C	D	Angle/°
F	C6	C7	C8	-179.2(4)	C8	N2	C11	C12	171.7(4)
O1	C1	C2	N1	57.2(6)	C8	N2	C13	O2	178.1(4)
O2	C12	C14	N3	-174.7(4)	C8	N2	C13	O3	-1.5(9)
O2	C12	C14	C16	-62.6(5)	C8	C9	C10	C5	0.2(7)
N1	C3	C4	O1	-59.7(6)	C10	C5	C6	F	179.9(4)
N1	C5	C6	F	1.5(7)	C10	C5	C6	C7	0.6(7)
N1	C5	C6	C7	-177.8(5)	C11	N2	C8	C7	16.0(7)
N1	C5	C10	C9	177.6(5)	C11	N2	C8	C9	-162.2(5)
N2	C8	C9	C10	178.7(4)	C11	N2	C13	O2	7.1(6)
N2	C11	C12	O2	20.1(5)	C11	N2	C13	O3	-172.5(5)
N2	C11	C12	C14	138.1(4)	C11	C12	C14	N3	69.9(5)
N3	C14	C16	O4	-15.3(5)	C11	C12	C14	C16	-178.0(4)
C1	O1	C4	C3	60.3(6)	C12	O2	C13	O3	-173.1(5)
C2	N1	C3	C4	56.3(5)	C12	O2	C13	N2	7.3(6)
C2	N1	C5	C6	-62.3(6)	C12	C14	C16	O4	-132.5(4)
C2	N1	C5	C10	119.5(5)	C13	O2	C12	C11	-17.5(5)
C3	N1	C2	C1	-55.5(6)	C13	O2	C12	C14	-139.7(4)
C3	N1	C5	C6	170.0(4)	C13	N2	C8	C7	-154.0(5)
C3	N1	C5	C10	-8.3(7)	C13	N2	C8	C9	27.8(8)
C4	O1	C1	C2	-58.7(6)	C13	N2	C11	C12	-17.1(6)
C5	N1	C2	C1	174.1(4)	C14	N3	C15	O4	-9.9(6)
C5	N1	C3	C4	-173.9(4)	C14	N3	C15	O5	171.3(5)
C5	C6	C7	C8	0.2(8)	C15	O4	C16	C14	10.9(6)
C6	C5	C10	C9	-0.8(7)	C15	N3	C14	C12	135.6(4)
C6	C7	C8	N2	-179.0(5)	C15	N3	C14	C16	15.7(5)
C6	C7	C8	C9	-0.8(7)	C16	O4	C15	O5	177.6(5)
C7	C8	C9	C10	0.6(7)	C16	O4	C15	N3	-1.3(6)

Table 25 Details of X-ray crystal structure determination of compound **92**.

4.3.3 Crystal structure analysis of compound 258



Crystal habitus	clear colourless needle	μ/mm^{-1}	0.993
Device type	Bruker D8-Venture	F(000)	736.0
Empirical formula	$C_{16}H_{18}FN_3O_5$	Crystal size/mm^3	0.14 x 0.05 x 0.04
Moiety formula	$C_{16}H_{18}FN_3O_5$	Absorption correction	Empirical
Formula weight	351.33	Tmin; Tmax	0.5659; 0.7536
Temperature/K	100	Radiation	CuKα (λ = 1.54178)
Crystal system	Orthorhombic	2Θ range for data collection/^{o}	11.708 to 135.488^o
Space group	$P2_12_12_1$	Completeness to theta	0.994
a/Å	6.5424(8)	Index ranges	- 7 ≤ h ≤ 7, -9 ≤ h ≤ 9, -36 ≤ h ≤ 37
b/Å	7.7850(10)	Reflections collected	17286
c/Å	31.526(5)	Independent reflections	2905 [R_{int} = 0.1592, R_{sigma} = 0.0965]
α/^{o}	90	Data/restraints/parameters	2905/0/226
β/^{o}	90	Goodness-of-fit on F^2	1.053
γ/^{o}	90	Final R indexes [I>=2σ (I)]	R_1 = 0.0624, wR_2 = 0.1383
Volume/Å^3	1605.7(4)	Final R indexes [all Data]	R_1 = 0.0950, wR_2 = 0.1572
Z	1	Largest diff. peak/hole/ e Å^{-3}	0.45/-0.48
ρ_{calc}g/cm^3	1.453	Flack parameter	0.3(2)

Table 26 Details of X-ray crystal structure determination of compound **258**.

Atom	Atom	Length/Å	Atom	Atom	Atom
F	C9	1.372(6)	N3	C10	F
O1	C2	1.449(6)	N3	C13	O1
O1	C3	1.367(7)	N3	C16	O1
O2	C3	1.205(6)	C1	C2	O2
O3	C4	1.455(5)	C1	C4	O3
O3	C5	1.372(7)	C4	C6	O3
O4	C5	1.210(6)	C7	C8	O4
O5	C14	1.438(7)	C7	C12	O5
O5	C15	1.426(6)	C8	C9	O5
N1	C1	1.451(7)	C9	C10	N1
N1	C3	1.357(7)	C10	C11	N1
N2	C5	1.361(7)	C11	C12	N2
N2	C6	1.467(6)	C13	C14	N2
N2	C7	1.423(7)	C15	C16	N2

Table 27 Details of X-ray crystal structure determination of compound **258**.

Atom	Atom	Atom	Angle/˚	Atom	Atom	Atom	Angle/˚
C3	O1	C2	109.0(4)	O4	C5	O3	121.1(5)
C5	O3	C4	109.6(4)	O4	C5	N2	129.2(6)
C15	O5	C14	110.2(4)	N2	C5	O3	109.7(4)
C3	N1	C1	111.6(5)	N2	C6	C4	101.8(4)
C5	N2	C6	111.4(4)	C8	C7	N2	120.7(5)
C5	N2	C7	126.8(4)	C12	C7	N2	119.9(5)
C7	N2	C6	121.6(4)	C12	C7	C8	119.4(5)
C10	N3	C13	114.8(4)	C9	C8	C7	118.0(5)
C10	N3	C16	114.8(4)	F	C9	C10	118.5(5)
C16	N3	C13	109.5(4)	C8	C9	F	116.4(5)
N1	C1	C2	100.4(4)	C8	C9	C10	125.0(5)
N1	C1	C4	112.4(5)	C9	C10	N3	120.0(5)
C4	C1	C2	114.9(4)	C11	C10	N3	125.5(5)
O1	C2	C1	104.9(4)	C11	C10	C9	114.4(5)
O2	C3	O1	121.3(5)	C12	C11	C10	122.9(5)
O2	C3	N1	129.3(6)	C11	C12	C7	120.3(5)
N1	C3	O1	109.4(4)	N3	C13	C14	110.5(4)
O3	C4	C1	110.2(4)	O5	C14	C13	109.4(5)
O3	C4	C6	104.9(4)	O5	C15	C16	111.6(5)
C1	C4	C6	112.2(4)	N3	C16	C15	110.9(5)

Table 28 Details of X-ray crystal structure determination of compound **258.**

A	B	C	D	Angle/˚	A	B	C	D	Angle/˚
F	C9	C10	N3	-0.8(7)	C5	N2	C7	C8	10.5(8)
F	C9	C10	C11	175.6(5)	C5	N2	C7	C12	-170.9(5)
O3	C4	C6	N2	15.7(5)	C6	N2	C5	O3	6.2(6)
O5	C15	C16	N3	-56.5(6)	C6	N2	C5	O4	-173.3(6)
N1	C1	C2	O1	-20.4(5)	C6	N2	C7	C8	-176.0(4)
N1	C1	C4	O3	-58.4(6)	C6	N2	C7	C12	2.6(7)
N1	C1	C4	C6	58.1(5)	C7	N2	C5	O3	-179.8(4)
N2	C7	C8	C9	178.4(4)	C7	N2	C5	O4	0.7(9)
N2	C7	C12	C11	179.9(4)	C7	N2	C6	C4	171.8(4)
N3	C10	C11	C12	177.1(5)	C7	C8	C9	F	-176.0(4)
N3	C13	C14	O5	59.4(6)	C7	C8	C9	C10	2.3(8)
C1	N1	C3	O1	-12.7(6)	C8	C7	C12	C11	-1.5(8)
C1	N1	C3	O2	168.0(5)	C8	C9	C10	N3	-179.1(5)
C1	C4	C6	N2	-103.9(4)	C8	C9	C10	C11	-2.7(8)
C2	O1	C3	O2	177.3(5)	C9	C10	C11	C12	0.9(8)
C2	O1	C3	N1	-2.1(6)	C10	N3	C13	C14	172.8(4)
C2	C1	C4	O3	55.5(6)	C10	N3	C16	C15	-174.9(4)
C2	C1	C4	C6	172.0(4)	C10	C11	C12	C7	1.1(8)
C3	O1	C2	C1	14.8(5)	C12	C7	C8	C9	-0.1(8)
C3	N1	C1	C2	20.6(5)	C13	N3	C10	C9	-61.3(6)
C3	N1	C1	C4	143.1(4)	C13	N3	C10	C11	122.8(5)
C4	O3	C5	O4	-175.5(5)	C13	N3	C16	C15	54.3(6)
C4	O3	C5	N2	5.0(5)	C14	O5	C15	C16	59.3(6)
C4	C1	C2	O1	-141.2(4)	C15	O5	C14	C13	-60.3(6)
C5	O3	C4	C1	107.6(5)	C16	N3	C10	C9	170.5(4)
C5	O3	C4	C6	-13.4(5)	C16	N3	C10	C11	-5.5(7)
C5	N2	C6	C4	-13.8(5)	C16	N3	C13	C14	-56.3(6)

Table 29 Details of X-ray crystal structure determination of compound **258**.

4.4 Headspace analysis from xylariaceous fungal strains

4.4.1 Total ion chromatograms of headspace extracts

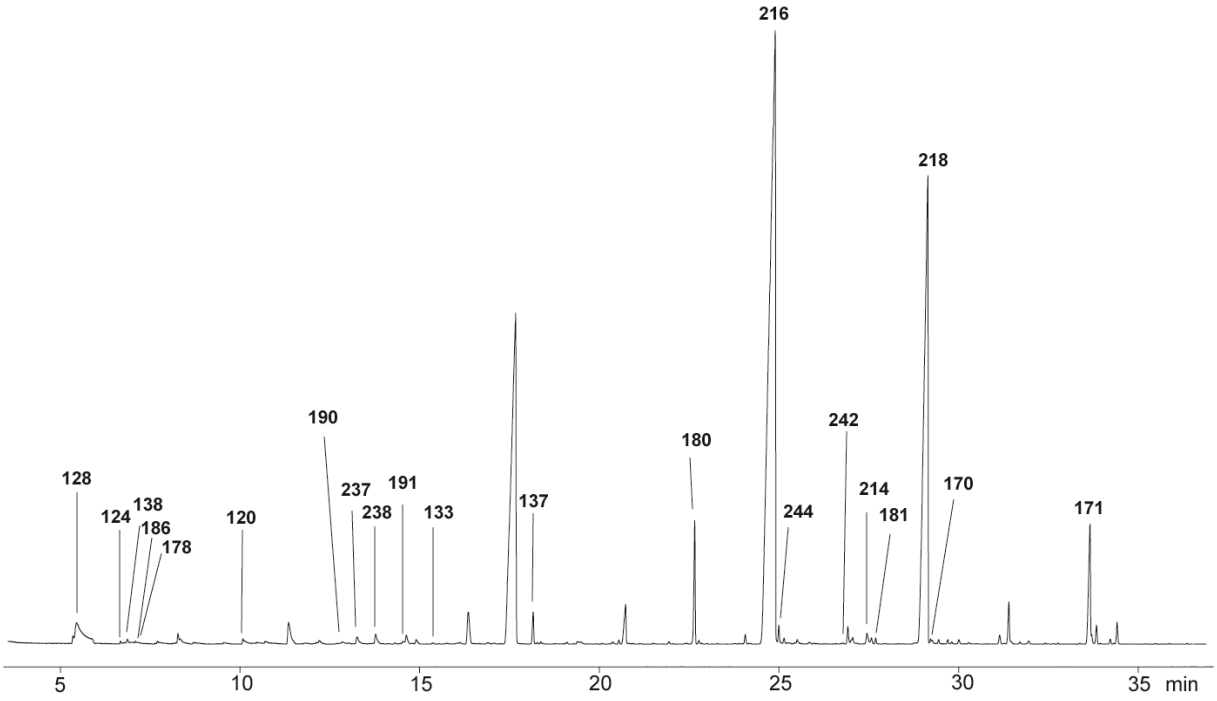


Figure 44 Total ion chromatograms of headspace extracts from fungal strain *Daldinia childiae* MUCL 53761.

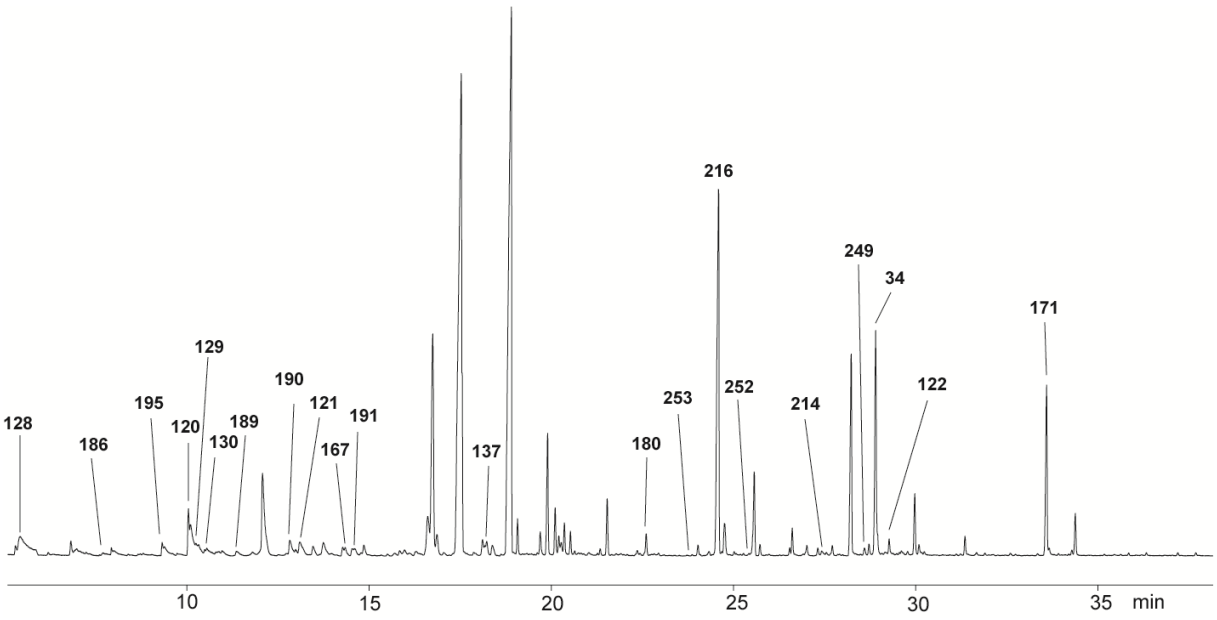


Figure 45 Total ion chromatograms of headspace extracts from fungal strain *Daldinia australis* CBS 119013.

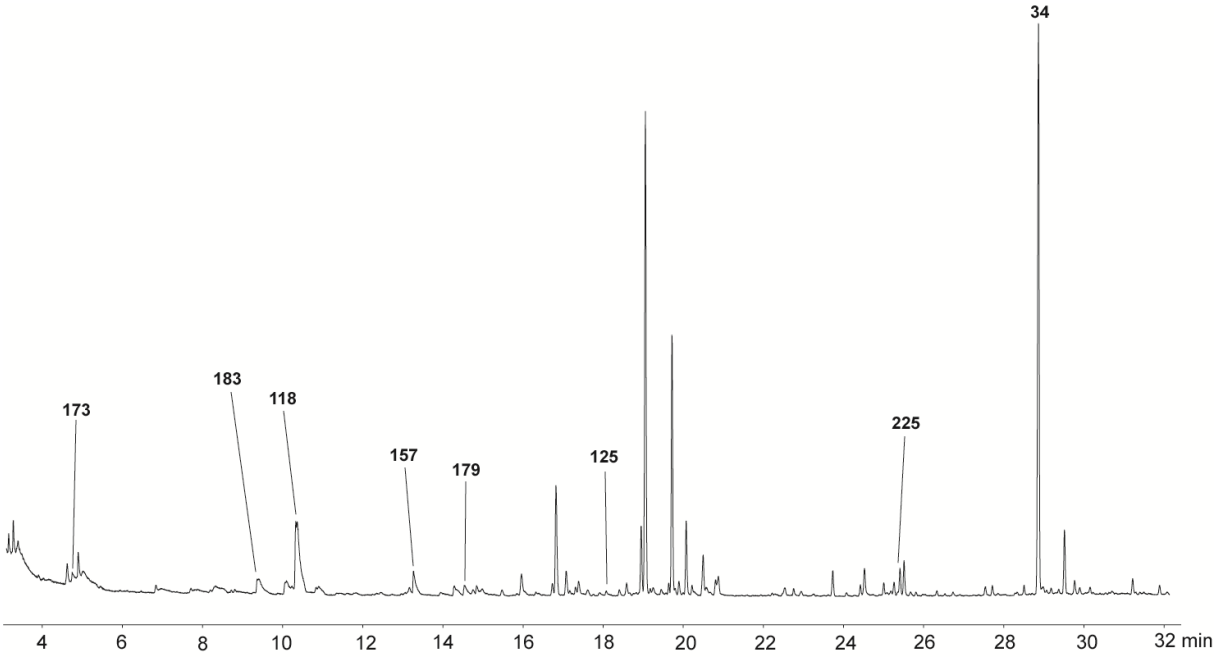


Figure 46 Total ion chromatograms of headspace extracts from fungal strain *Daldinia cf. caldarium* CBS 113045.

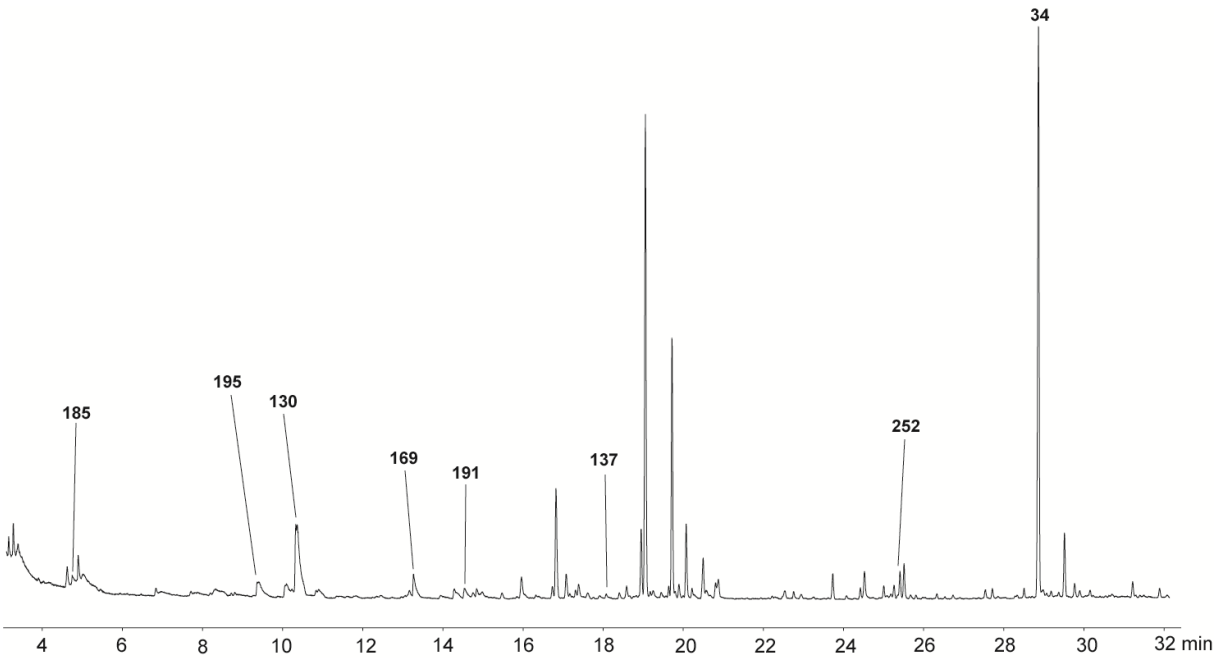


Figure 47 Total ion chromatograms of headspace extracts from fungal strain *Daldinia eschscholzii* STMA 11017.

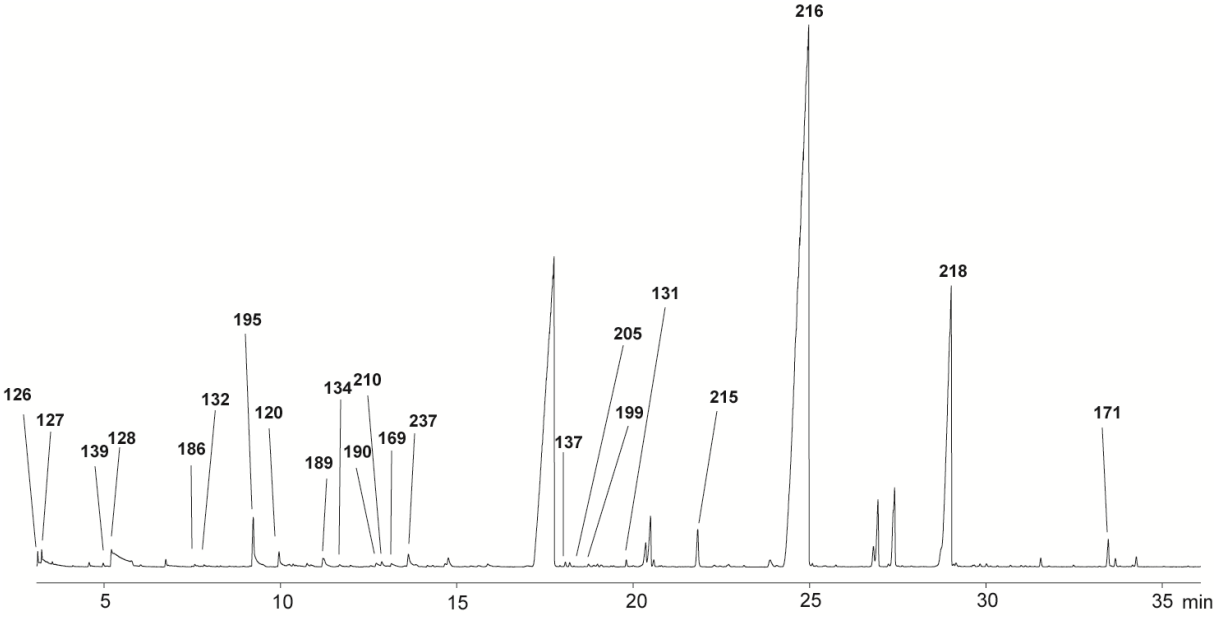


Figure 48 Total ion chromatograms of headspace extracts from fungal strain *Daldinia novae-zelandiae* STMA 05243.

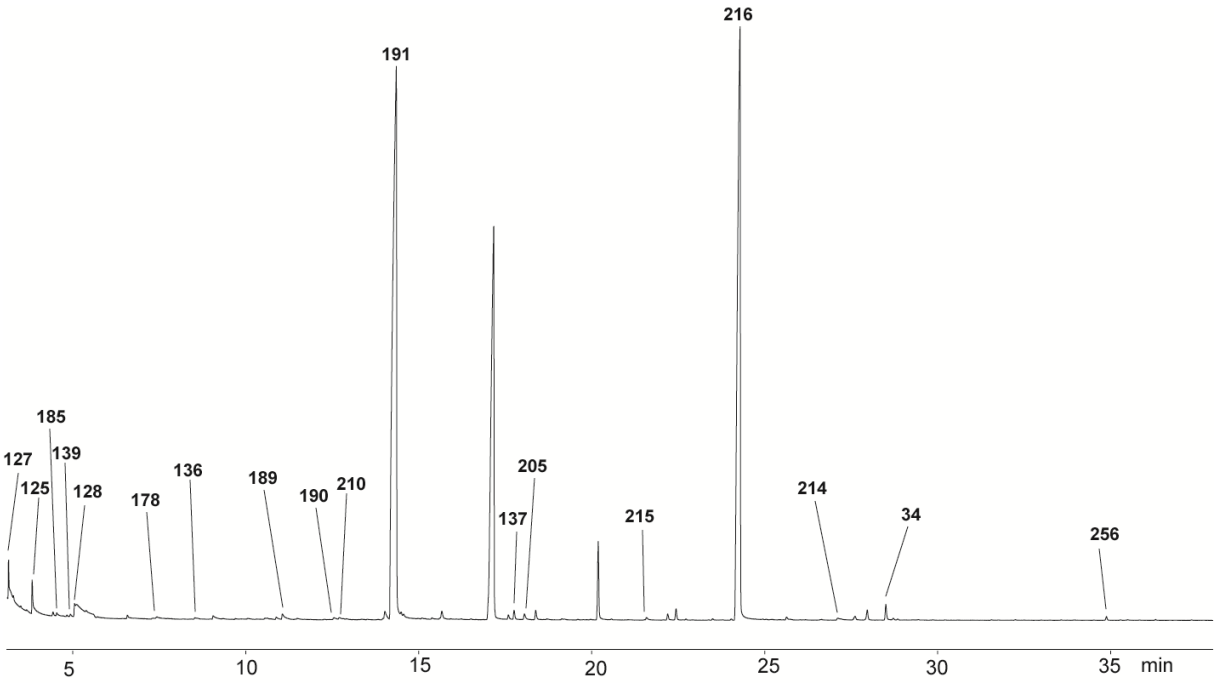


Figure 49 Total ion chromatograms of headspace extracts from fungal strain *Daldinia hawksworthii* STMA 13010.

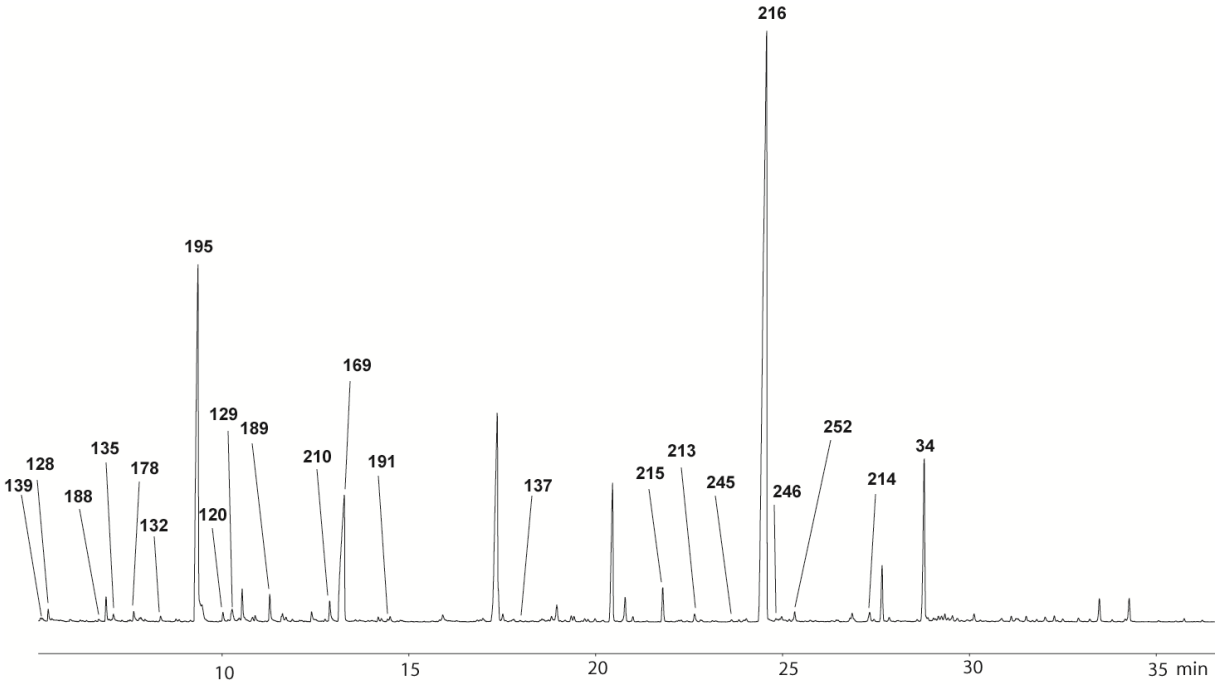


Figure 50 Total ion chromatograms of headspace extracts from fungal strain *Daldinia concentrica* STMA 05061.

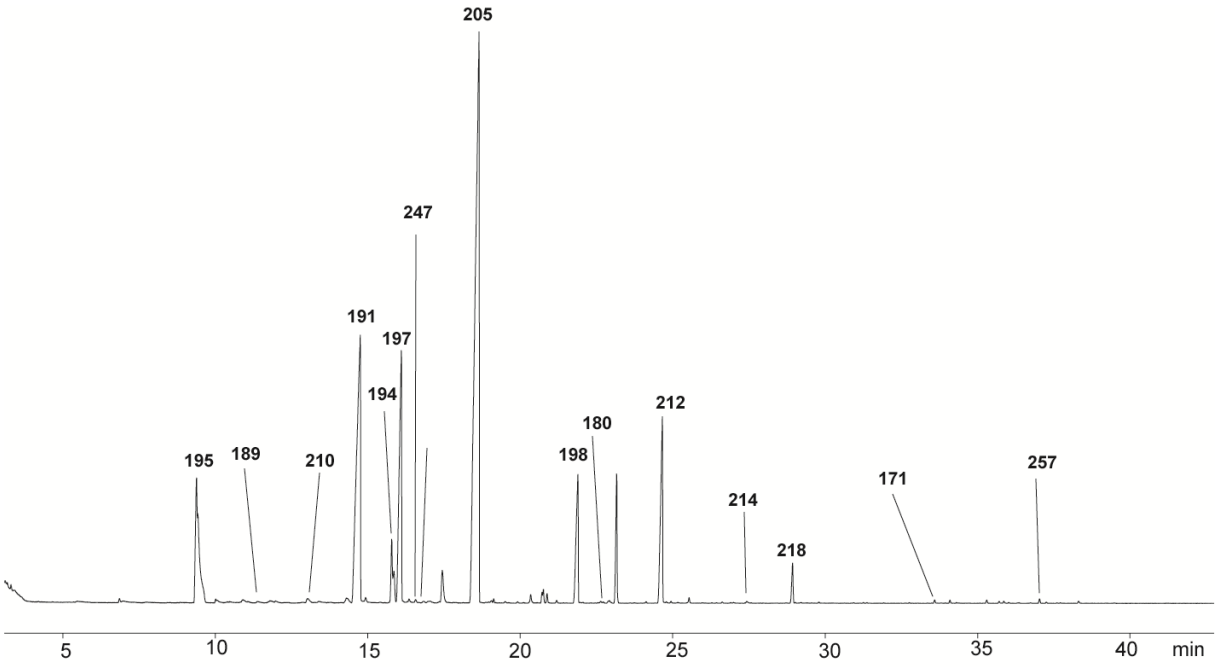


Figure 51 Total ion chromatograms of headspace extracts from fungal strain *Hypoxylon* sp. nov. JF 11167.

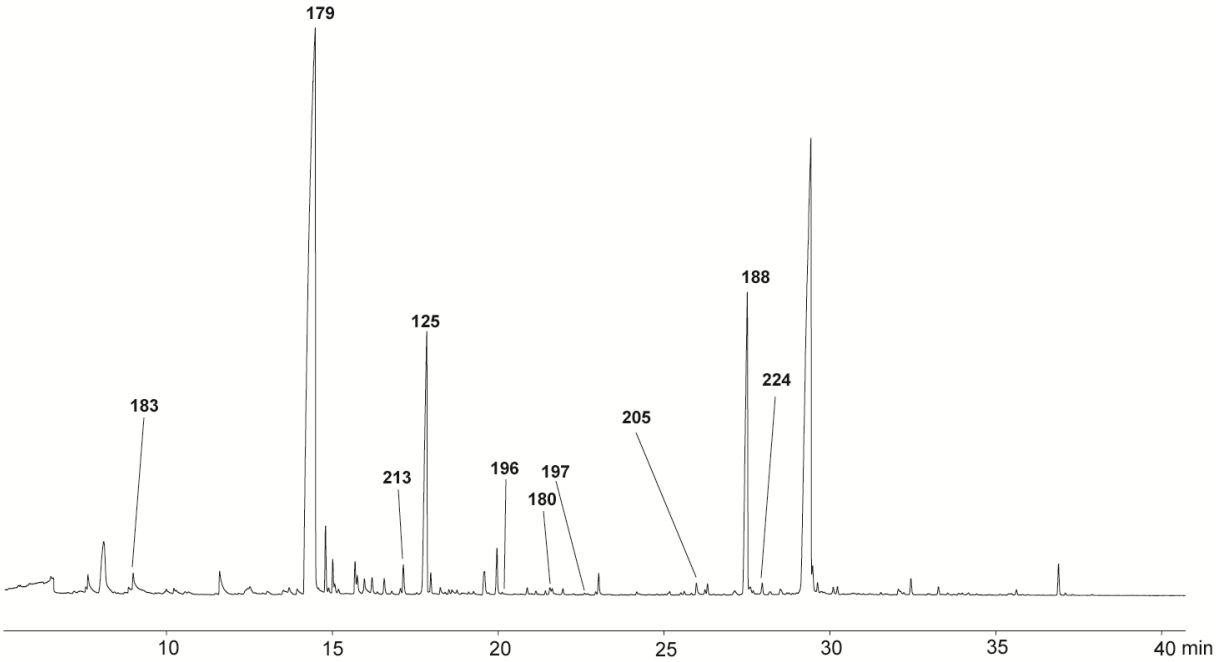


Figure 52 Total ion chromatograms of headspace extracts from fungal strain *Hypoxylon* sp. nov. STMA 11183.

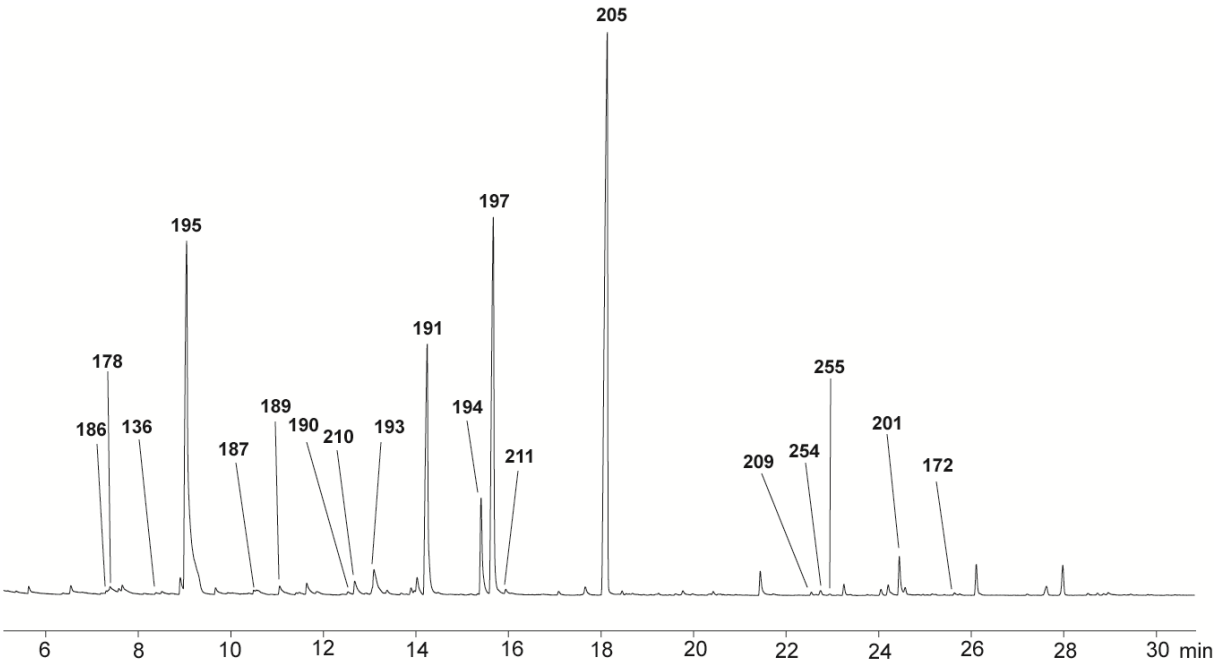


Figure 53 Total ion chromatograms of headspace extracts from fungal strain *Hypoxylon macrocaipum* STMA 05121.

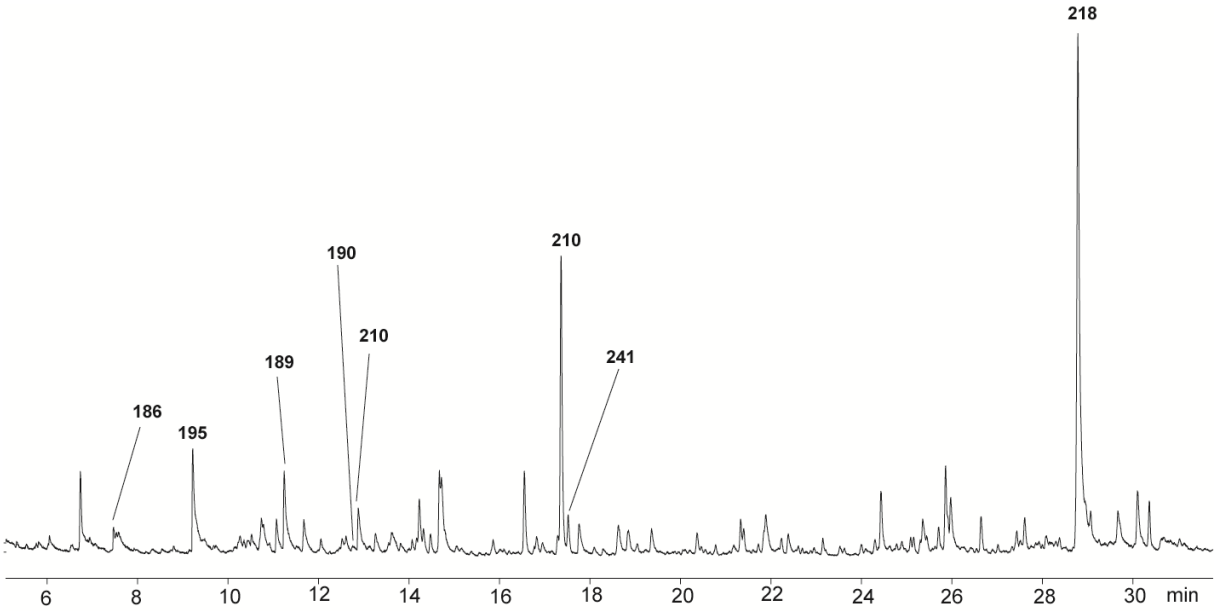


Figure 54 Total ion chromatograms of headspace extracts from fungal strain *Hypoxylon griseobrunneum* STMA 10235.

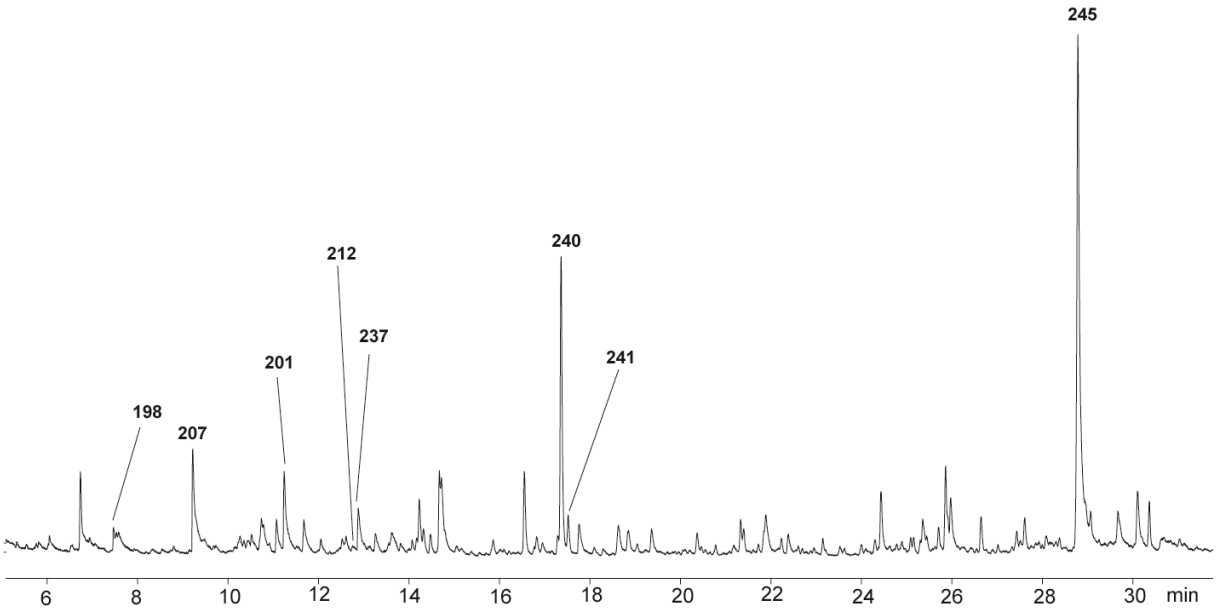


Figure 55 Total ion chromatograms of headspace extracts from fungal strain *Hypoxylon rubiginosum* STMA 03027.

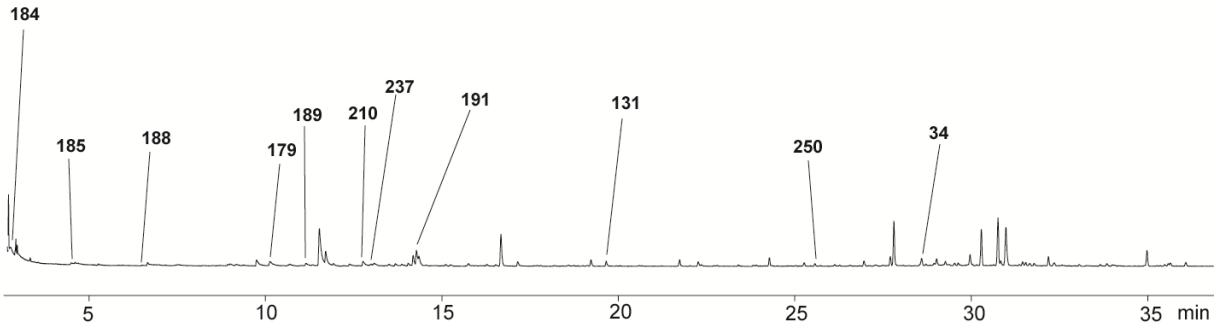


Figure 56 Total ion chromatograms of headspace extracts from fungal strain *Biscogniauxia cylinderispora* STMA 12118.

4.4.2 Summary of volatile organic compounds

Compound	*I*	*I* (Lit.)	Ident.	Occurrence in strain
Pyrazine (**184**)		737[479]	ms	1, 14
Tiglic aldehyde (**126**)		749[480]	ms	1,6
2-Methyl-3-pentanone (**127**)		742[481]	ms	1, 6, 7
2,3-Butanediol (**125**)		795[479]	ms	7
2-Hexanol (**123**)	811	813[482]	ms, ri	1
Butyl acetate (**166**)	823	823[483]	ms, ri	1
Methylpyrazine (**185**)	829	827[484]	ms, ri	1, 4, 7, 13, 14
Acetyl valeryl (**139**)	838		ms	1, 6, 7, 8
4-Methyl-3-hexanone (**128**)	845	842[485]	ms, ri	1, 2, 3, 6, 7, 8
Cyclohexanol (**124**)	886	880[486]	ms, ri	2
2,6-Dimethylpyridine (**188**)	888	887[487]	ms, ri	8
Cyclohexanone (**138**)	899	903[488]	ms, ri	2, 14
2-Hydroxyhexan-3-one (**135**)	901	896[489]	ms, ri	1, 8
2,5-Dimethyl-pyrazine (**186**)	913	911[490]	ms, ri	1, 2, 3, 6, 11, 12
1-(2-Furanyl)-ethanone (**178**)	916	915[491]	ms, ri	1, 2, 7, 8, 11
(*E*)-4-Methylhex-4-en-3-one (**132**)	936		ms, ri	1, 6, 8
2-Hydroxy-4-methylhexan-3-one (**136**)	943		ms, ri	1, 5, 7, 11
Ethyl 3-hydroxy-3-methylbutanoate (**168**)	960	955[492]	ms, ri	14
Benzaldehyde (**196**)	962	964[479]	ms, ri	3,8

1-Octen-3-ol (**120**)	981	981[493]	ms, ri	1, 2, 3, 5, 6, 8
3-Octanone (**129**)	988	987[493]	ms, ri	1, 3, 4, 5, 8
6-Methyl-5-hepten-2-one (**134**)	988	988[494]	ms, ri	6
2-Acetyl-5-methylfuran (**179**)	993	987[495]	ms, ri	14
2-Octanone (**130**)	994	994[496]	ms, ri	3
Trimethylpyrazine (**187**)	1004	1005[491]	ms, ri	11
2-Acetylthiazole (**189**)	1018	1021[491]	ms, ri	1, 3, 6, 7, 8, 9, 11, 12, 13, 14
2-Hydroxybenzaldehyde (**196**)	1044	1040[497]	ms, ri	5
α-Methyl benzenemethanol (**190**)	1061	1063[498]	ms, ri	1, 2, 3, 6, 7, 11, 12
Acetonphenone (**210**)	1064	1061[499]	ms, ri	1, 5, 6, 7, 8, 9, 11, 12, 13, 14
(*E*)-2-Octen-1-ol (**121**)	1070	1067[500]	ms, ri	3, 5
6-Methyl-5,6-dihydro-2*H*-pyran-2-one (**169**)	1072	1074	ms, ri, std	1, 4, 5, 6, 8
3-Methyl-phenol (**193**)	1080	1075[501]	ms, ri	11
cis-Linalool oxide, furanoid (**237**)	1086	1067[502]	ms, ri	1, 2, 6, 13, 14
trans-Linalool oxide, furanoid (**238**)	1089	1089[503]	ms, ri	2
Hexyl propionate (**167**)	1107	1105[504]	ms, ri	3
Phenylethyl alcohol (**191**)	1113	1113[505]	ms, ri	2, 3, 4, 7, 8, 9, 10, 11, 14
Manicone (**133**)	1136	1140	ms, ri, std	1, 2
1,2-Dimethoxybenzene (**204**)	1150	1150[506]	ms, ri	13
2,5-Dimethyl-phenol (**194**)	1155	1148[507]	ms, ri	9, 11
2-Hydroxy-4-methylbenzaldehyde (**197**)	1163	1170	ms, ri, std	9, 11
1-Phenyl-1,2-propanedione (**211**)	1170	1175[508]	ms, ri	11
Terpinene-4-ol (**247**)	1178	1178[509]	ms, ri	9
2-Methoxy-5-methylphenol (**203**)	1187	1191[510]	ms, ri	9
cis-Linalool oxide, pyranoid (**239**)	1187	1186[511]	ms, ri	1, 2, 14
Methyl 2-hydroxybenzoate (**212**)	1195	1195[503]	ms, ri	5
2-Hydroxy-1,8-cineole (**240**)	1210	1219[512]	ms	10, 12
1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-6-one (**241**)	1215	1217[503]	ms, ri	12

(4*R*,5*R*,6*S*)-5-Hydroxy-4,6-dimethyloctan-3-one (**137**)	1239	1241	ms, ri, std	1, 2, 3, 4, 6, 7, 8, 10
3,4-Dimethoxytoluene (**205**)	1239	1242	ms, ri, std	6, 7, 9, 11, 13
2,5-Dimethoxytoluene (**206**)	1251	1243[513]	ms, ri	13
4-Methoxybenzaldehyde (**199**)	1253	1250[514]	ms, ri	6
2-Undecanone (**131**)	1291	1291[514]	ms, ri	6
1,2,3-Trimethoxybenzene (**208**)	1315	1315[515]	ms, ri	10, 13
2-Methoxy-4-methylbenzaldehyde (**198**)	1364	1364	ms, ri, std	9
2-Methyl-4-chromanone (**215**)	1366	1367	ms, ri, std	1, 6, 7, 8
2-(4-Methoxyphenyl)ethanol (**192**)	1369	1374[516]	ms, ri	10
2-Nonylfuran (**180**)	1391	1399	ms, ri, std	1, 2, 3, 9
1-(2-Hydroxy-6-methoxyphenyl)ethanone (**213)**	1397	1402[517]	ms, ri	8
1,2,3-Trimethoxy-5-methyl-benzene (**209**)	1412	1410[518]	ms, ri	10, 11, 13
α-Cedrene (**254**)	1415	1411[519]	ms, ri	11
β-Cedrene (**255**)	1423	1423[520]	ms, ri	11
Dihydro-β-ionone (**245**)	1437	1433[521]	ms, ri	8
β-Barbatene (**253**)	1446	1440[522]	ms, ri,	3
1,2-Dimethoxy-4-(methoxymethyl)benzene (**207**)	1452	1452[523]	ms	13
2,5-Dimethoxy-benzaldehyde (**202**)	1460		ms	13
4,7-Dihydroxy-2*H*-1-benzopyran-2-one (**216**)	1469		ms	1, 2, 3, 6, 7, 8, 9
3,4-Dimethoxy-benzaldehyde (**201**)	1483		ms	11
trans-β-Ionone (**246**)	1484	1485[524]	ms, ri	8
trans-β-Bergamotene (**244**)	1489	1480[525]	ms, ri	2
γ-Curcumene (**243**)	1490	1485[526]	ms, ri	1
α-Bulnesene (**252**)	1508	1508[527]	ms, ri	3, 4, 8
δ-Cadinene (**250**)	1529	1529[528]	ms, ri	14
Dihydroactinidiolide (**172**)	1533	1531[503]	ms, ri	11
Mellein (**217**)	1547		ms, ri	10
Nerolidol (**242**)	1565	1565[529]	ms, ri	2

5-Hydroxy-2-methyl-4*H*-chromen-4-one (**214**)	1593	1592	ms, ri, std	2, 3, 7, 9, 8
2-Undecylfuran (**181**)	1603		ms	2
2,4-Dimethoxy-6-methyl-benzaldehyde (**200**)	1613		ms	10
α-Acorenol (**251**)	1633	1632[530]	ms, ri	10, 13
T-Muurolol (**249**)	1645	1645[531]	ms, ri	3
Pogostol (**34**)	1653	1656[532]	ms, ri	1, 3, 4, 5, 7, 8, 14
6-Heptyl-2*H*-pyran-2-one (**170**)	1665		ms	1, 2
1,8-Dimethoxynaphthalene (**218**)	1668		ms	2, 9
α-Bisabolol (**248**)	1676	1675[533]	ms, ri	13
1-Tetradecanol (**122**)	1676	1676[534]	ms, ri	3
6-Nonyl-2*H*-pyran-2-one (**171**)	1880	1889	ms, ri, std	1, 2, 3, 6, 9
8(14),15-Pimaradiene (**256**)	1968	1942[535]	ms, ri	7
Abietatriene (**257**)	2060	2055[536]	ms, ri	9

Table 30 Organic volatile compounds released by the Xylariaceae family. Identification based on comparison of mass spectrum to a data base spectrum (ms), comparison of retention index of a published retention index on the same or a similar GC fused silica capillary column (ri), and comparison to a synthetic or commercially available referefence compound (std).

ID	Strain
1	*Daldinia clavata* STMA 06094
2	*Daldinia childiae* MUCL 53761
3	*Daldinia australis* CBS 119013
4	*Daldinia cf. caldarium* CBS 113045
5	*Daldinia eschscholzii* STMA 11017
6	*Daldinia novae-zelandiae* STMA 05243
7	*Daldinia hawksworthii* STMA 13010
8	*Daldinia concentrica* STMA 05061
9	*Hypoxylon* sp.nov. JF 11167
10	*Hypoxylon* sp.nov. STMA 11183
11	*Hypoxylon macrocaipum* STMA 05121
12	*Hypoxylon griseobrunneum* STMA 10235
13	*Hypoxylon rubiginosum* STMA 03027
14	*Biscogniauxia cylinderispora* STMA 12118

Table 31 ID number of the investigated Xylariaceae fungal strains.

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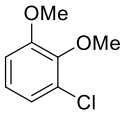
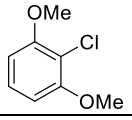
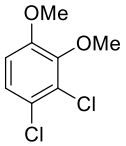
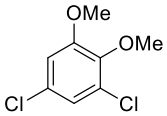
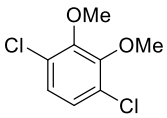
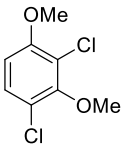
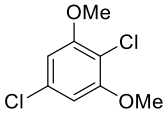
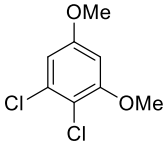
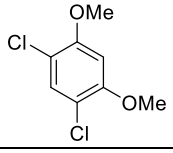
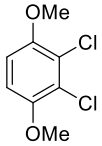
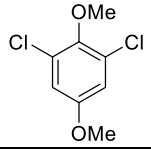
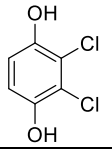
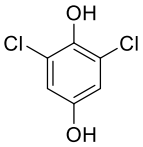
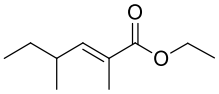
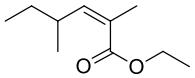
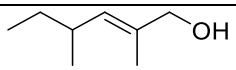
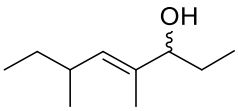
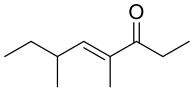
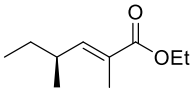
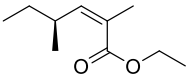
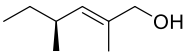
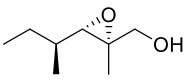
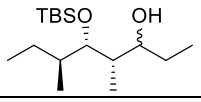
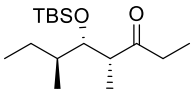
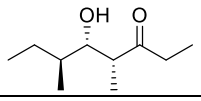
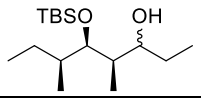
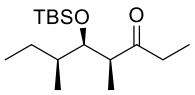
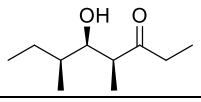
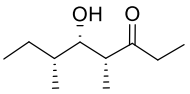
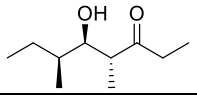
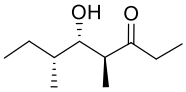
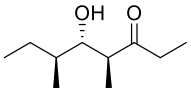
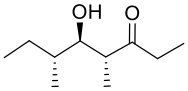
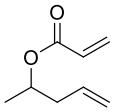
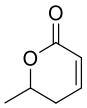
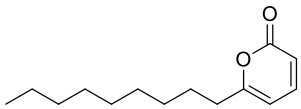
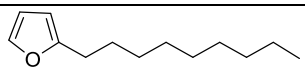
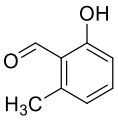
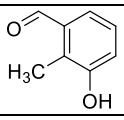
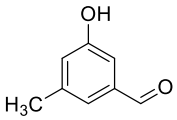
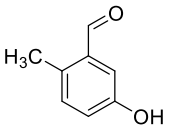
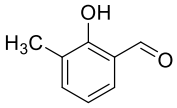
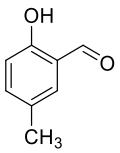
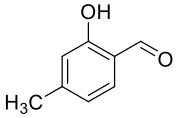
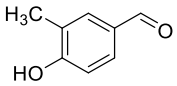
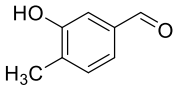
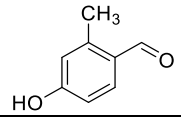
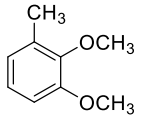
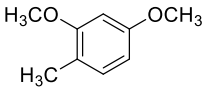
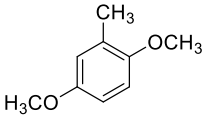
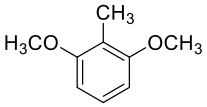
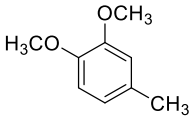
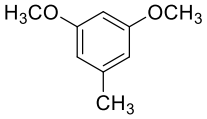
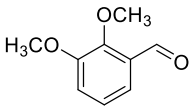
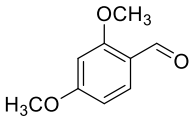
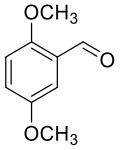
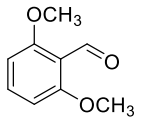
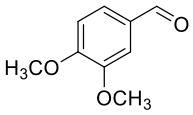
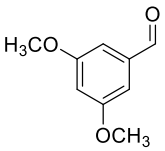
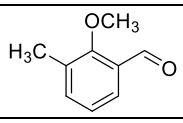
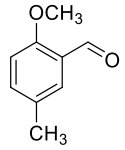
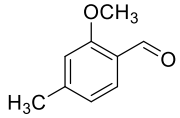
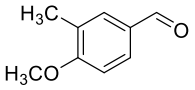
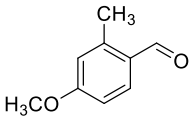
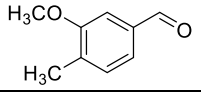
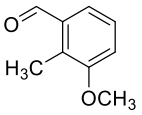
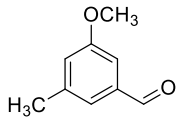
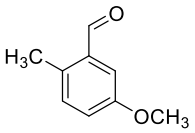
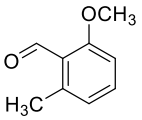
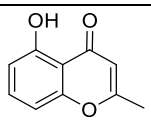
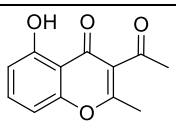
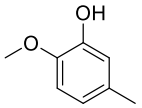
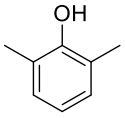
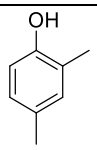
Appendix

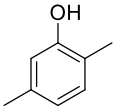
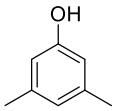
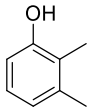
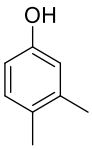
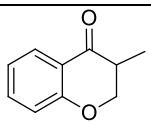
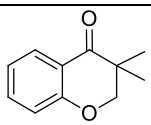
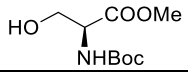
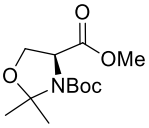
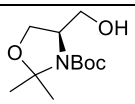
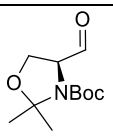
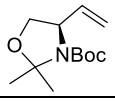
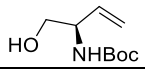
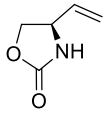
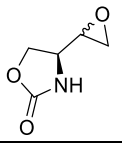
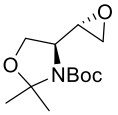
Number	Compound	GC-MS	NMR
105a		TWN0088	wao121569
105c		TWN0075	wao121148
106a		TW0220 TWN0060	wao120843
106b		TW0174 TWN0034	wao120471
106c		TW0217 TWN0058	wao120827
106e		TW0157 TWN0023	wao120577
106f		TW0155 TWN0021	wao120260
106g		TW0154 TWN0018	wao120252
106h		TW0131 TWN0022	wao120023
106i		TW0351 TWN0094	wao121710
106k		TW0177 TWN0021	wao120513

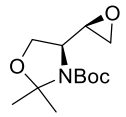
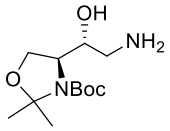
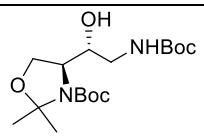
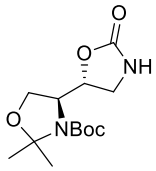
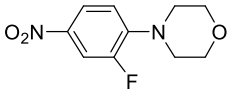
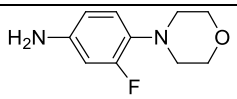
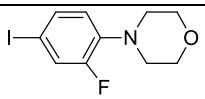
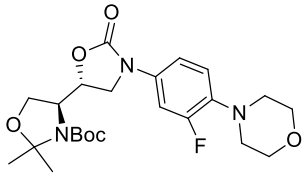
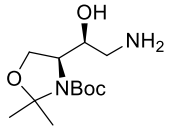
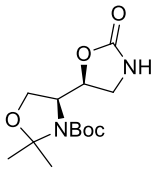
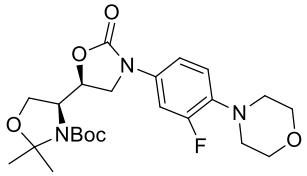
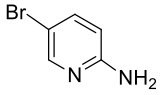
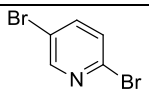
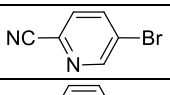
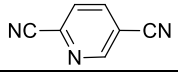
117		TW0168	wao120459
119		TW0167	wao120458
***E*-142**		TW0553	wao121309
***Z*-142**		TW0550	wao121290
143		TW0555	wao124345
144		TW0556	wao123760
133		TW0557	wao121972
***E*-148**		TW0425	wao122218
***Z*-148**		TW1403	wao122256
149		TW0444	wao122257
150a		TW0453	wao122701
152a		TW1389	wao123973
153a		TW0515	wao123985
(4*R*,5*S*,6*S*)-137a		TW1276	wao124019
150b		TW0430	wao122522
152b		TW0508	wao123191
153b		TW0509	wao123986
(4*S*,5*R*,6*S*)-137b		TW1277	48c5a044.16 49c5a009.16
(4*R*,5*S*,6*R*)-137b		TW1278	48c5a043.16 49c5a008.16

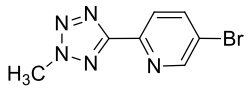
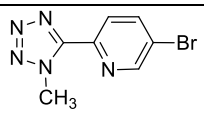
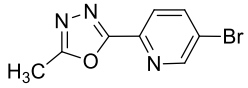
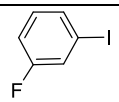
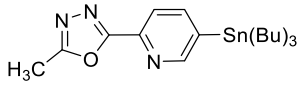
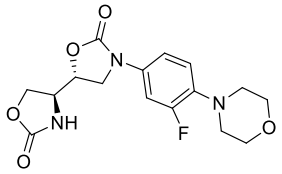
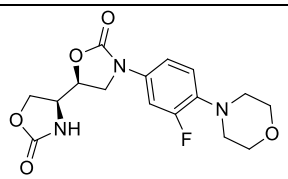
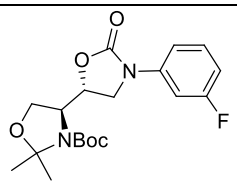
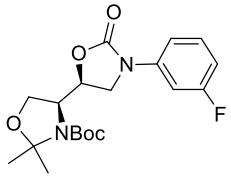
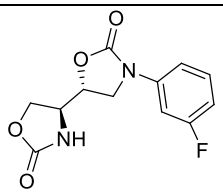
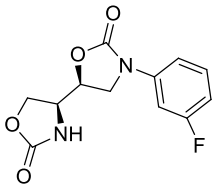
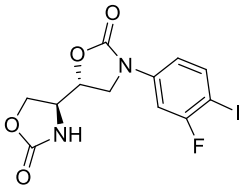
(4*R*,5*R*,6*S*)-137d		TW1269	49c5a045.16 50p5a019.16
(4*S*,5*S*,6*R*)-137d		TW1266	49c5a044.16 50p5a012.16
(4*S*,5*S*,6*S*)-137c		TW1267	49c5a047.16 49c5a009.16
(4*R*,5*R*,6*R*)-137c		TW1268	49c5a046.16 50c5a008.16
175		TW0281	wao121278
169		TW0397 TWN0105	wao122136
171		TWN0682	wao121382
180		TW1404	wao120182
		TW1425	
		TW1422	
		TW1423	
		TW1424	
		TW1369	
		TW1368	
197		TW1379	
		TW1367	
		TW1416	

		TW1415	
		TW1427	
		TW1366	
		TW1428	
		TW1429	
205		TW1377	
		TW1364	
		TW1430	46p5a057.17
		TW1431	43t4a010.17
		TW1432	43t4a012.17
		TW1433	
		TW1365	
		TW1434	
		TW1380	

		TW1384	
198		TW1382	
		TW1383	43t4a011.17
		TW1435	46c5a024.17
		TW1421	46c5a001.17
		TW1438	46c5a005. 7
		TW1439	46c5a003.17
		TW1440	46c5a004.17
		TW1441	46c5a005.17
214		TW0400 TWN0117	wao122165
220		TW0402 TWN0118	wao122194
		TW1443	
		TW1355	
		TW1353	

194		TW1354	
		TW1359	
		TW1352	
		TW1356	
215		TW1343	10c5a034.17
		TW1342	10c5a033.17
264			36x3a014.14
265		TW0572 TW0573	36x3a017.14
266		TW0579	30x3a060.15
260		TW0580	38x3a066.14
267		TW0595	02x4a108.15
268		TW0598	40x4a014.14
269		TW0603	48x4b052.14
270		TW0614	43x3a020.14
280		TW1027	36x4a049.15

281		TW1028	36x4a050.15
282			14x3a021.15
283			08p5a027.16
278		TW1106	39x4a045.15
275		TW0578	36x3a010.14
72		TW720	38x3a020.14
279		TW0808	24x3b039.15
293			19m5a018.15
295			38x3a002.15
296		TW1093	13p5a039.16
297			29c5a022.16 14c5a016.17
307		TW1085	10p5a028.16
308		TW1086	12p5a004.16
309		TW1094	13t4a092.16
		TW1123	16p5b018.16

311		TW1150	20c5a045.16
312		TW1149	20c5a046.16
313		TW1131	22p5a029.16
301		TW1119	17p5a054.16
320			05p5a014.17
92			07p5a046.16 08p5a010.16 07l3a027.16
258			29c5boo4.16 14c5a033.17
304			16p5b020.16 16c5a041.16
321			07c5a011.17
306			20c5a044.16
323			11c5a047.17
299			13c5a031.17

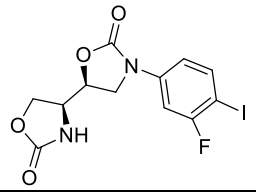
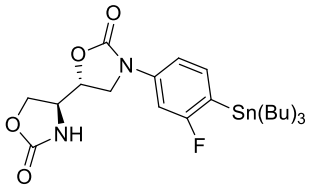
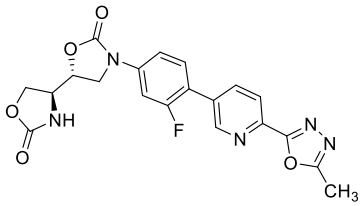
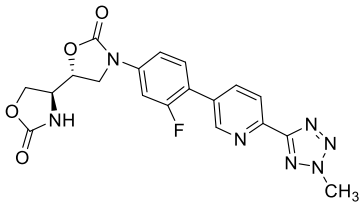
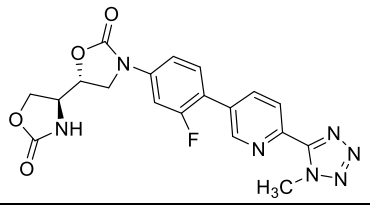
324			12c5a027.17
319			13c5a007.17
102			06p5a034.17 14c5a007.17
103			07s7a007.17 12c5a010.17
104			07p5a019.17 12c5a011.17

Table 32 Analytical data of synthetic samples and commercially available chemicals.